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14. ABSTRACT
STRIVE is a story-driven approach to using virtual reality (VR) for understanding and training psychological resilience in service members prior to a combat deployment. This effort is based on two scientific principles: 1) pre-exposure to traumatic events within a safe environment provides some degree of protection for those later exposed to subsequent trauma and 2) resilience, or the rate and effectiveness with which someone returns to normal after stress, can be strengthened through systematic training. This project created the fifth episode (of six) of STRIVE and also resulted in four papers.

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STRIVE, Allostatic Load, Virtual Reality, Mentor, PTSD, Psychological Resilience

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FINAL TECHNICAL REPORT

The STRIVE-ONR Project: Stress Resistance in Virtual Environments

Period of Performance: May 2012 – April 2015

Award Number: N00014-12-1-0163

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Background

The STRIVE program is designed as an interactive/immersive narrative-based set of VR episodes that expose participants to events that have been reported by SMs with PTSD as emotionally challenging and in that context present resilience training skills and techniques by way of an Interactive Virtual Mentor (IVM). The project evolved from the Virtual Iraq/Afghanistan Virtual Reality Exposure Therapy (VRET) system at the University of Southern California Institute for Creative Technologies and aims to foster psychological resilience by creating a set of combat and ethically challenging simulations that can be used as contexts for the experiential learning of cognitive-behavioral emotional coping strategies in SMs prior to deployment. This process involves immersing and engaging SMs within a variety of virtual "missions" where they are confronted with emotionally challenging situations that are inherent in a combat environment. Interaction by SMs within such emotionally challenging scenarios aims to provide a more meaningful context in which to learn and practice psychoeducational and cognitive coping strategies that research suggests plays a supportive role in the psychological preparation for a combat deployment. To accomplish this, STRIVE has been designed as a multi-episode interactive narrative in VR, akin to being immersed within a "Band of Brothers" type storyline that spans a typical deployment cycle. We currently have an initial story arc of six episodes created that will be used in the research that is now being proposed. At the end of each of the graded 10-minute episodes, an emotionally challenging event occurs, designed in part from feedback provided by SMs undergoing PTSD VRET (e.g., seeing/handling human remains, direct threat to safety via an IED attack in a vehicle, moral challenges due to culturally relativistic events (wife beating), the death of a civilian child, death of a squad member, grief processing in theatre). At that point in the episode, the virtual world "freezes in place" and an IVM emerges from the midst of the chaotic VR scenario to guide the user through stress-related psychoeducational and self-management tactics, as well as provide rational restructuring exercises for appraising and processing the virtual experience. The resilience training component is drawing on evidence-based content that has been endorsed as part of standard classroom-delivered DoD resilience training programs, as well as content that has been successfully applied in non-military contexts (e.g., humanitarian worker training, sports psychology, etc.). In this fashion, STRIVE provides a digital "emotional obstacle course" that can be used as a tool for providing context-relevant learning of emotional coping strategies under very tightly controlled and scripted simulated conditions.

Scientific and Technical Approach

The STRIVE project involved a comprehensive design and development effort--employing screenwriting, interactive narration, graphic design, animation, virtual humans, natural language and interactive tutoring—all to support ongoing clinical and scientific goals. The design and development effort utilized an integrative effort of many departments at ICT to provide a state-of-the-art immersive experience. This experience, as developed for STRIVE, not only required the graphical development of a visually realistic environment, it utilized surround sound audio, high

quality voice acting and, perhaps most importantly, compelling dramatic narrative that fully engages the user in emotionally turbulent scenes. Finally, it developed a mentor character—with empathy, realism, and a certain macho fatalism that is the emotional currency of the military combined with the wisdom and understanding of a chaplain with a PhD in neuroscience—who can discuss the emotions experienced by the user during the preceding episode.

This psychologically savvy, artistically advanced episode was developed for two equally important purposes. First was to provide a clinically effective means of increasing stress resilience among pre-deployed warfighters. Clinical effectiveness cannot be fully demonstrated in a short-term study of such a novel treatment approach as is being developed by STRIVE. In essence, we proved the clinical appropriateness of these episodes by demonstrating user acceptance and effective psychophysiological responses. We firmly believe that demonstration of these clinical successes will lead to large scale, longitudinal studies of this interactive, highly cost effective training method, of the scale needed to fully establish efficacy. Our second purpose was to utilize the ability to control all visual and auditory stimuli possible in immersive environments to systematically understand how pre-deployed Soldiers and Marines respond on a psychophysiological basis to emotionally stressful episodes. By linking psychophysiological measurements to specific segments of episodes that have been validated to evoke certain stress-related emotions, we have gained better understanding of the psychophysiology of stress reactivity and stress resolution, and in so doing, we are positioning ourselves to be able to identify effective vs. dysfunctional stress reactions.

During all of the STRIVE virtual training experiences, warfighters are monitored physiologically as part of a larger investigation into the psychophysiology and the biomarkers of the stress response. One such construct, AL, is directly investigated via physiological and neuro-hormonal analysis from specimen collections taken immediately before engagement in the STRIVE virtual experience. Further, extensive psychophysiological measurements are recorded during all VR stress resilience episodes. Measurements include electrocardiograms (ECG), galvanic skin conductance (GSR), respiration rate and maximal respiration and electroencephalograms (EEG). During this phase of STRIVE where we focus on grief responses, we hypothesize that VR stress resilience training with warfighters will reduce the later incidence of PTSD and other psychosocial health conditions, specifically those associated with loss. As well, the new knowledge garnered from the physiological markers of AL will inform understanding of the resilience process when a warfighter processes, to greater and lesser extents, the death of a colleague. This project is an ideal merger between the development of an emotional coping training simulation application and a basic neurophysiological protocol for evolving more objective measurement of the response to stress and ultimately to resilience. A VR simulation in this sense offers the properties of an "ultimate Skinner Box" that presents a controlled, context relevant stimulus environment for producing the high level of consistency required to advance precision testing of stress reactions and inform and evolve our understanding of the construct of resilience.

Accomplishments/Results

The STRIVE-ONR project funded the development of episode 5 entitled "Loss of a Leader," and is very similar in structure to other episodes but includes the loss of the unit leader during a dismounted patrol. It also funded four papers, three of which report on the development and validation of STRIVE and the fourth which analyzes and reports on allostatic load in the Marine Resilience Study data. While the analyses were completed for this paper the principal author from the Marine Resilience Study was unavailable to complete this project. Thus, an additional STRIVE-based paper has been developed and a draft is included.

Additionally, under separate funding, episode 5 was used in testing a population of Colorado National Guardsmen before and after their deployment to Afghanistan. A total of 28 Guardsman were tested with STRIVE and also provided blood samples prior to testing as well as EKG and GSR during the STRIVE testing session.

Results from the Colorado National Guard study will be presented at the International Society for Traumatic Stress Studies (ISTSS) Conference in November 2015. Additionally, the USC Institute for Creative Technologies and the Samsung Corporation are in discussions on translating the STRIVE episodes into the Samsung Gear VR headset so that the STRIVE episodes may be viewed in a head-mounted display by SMs.

In 2014, STRIVE received the *U.S. Army Modeling and Simulation Award for Army Wide Training* as a component of the Squad Overmatch project.

The results obtained from the STRIVE research protocol continue to be analyzed and are the source of two dissertation projects. The four manuscripts that are attached contain many novel and groundbreaking findings including the following:

In conjunction with Drs. Bruce McEwen, Teresa Seeman and Arun Karamangla, Drs. Rizzo and Buckwalter completed the most advanced methodological study of allostatic load (AL) to date. Utilizing a factor analytic design followed by two machine learning algorithms (k-means clustering and self-organizing maps (SOM), the researchers identified 7 robust clusters that characterize AL. In terms of highlights: (a) our Healthy profile had few clinical problems, revealing a unique and positive view of biomarkers and health risk factors in combination; (b) Pro-Inflammatory 59% with high blood pressure and 36% diabetes; (c) Low Stress Hormones linked to heart disease, TIA/Stroke, diabetes and circulation problems; and (d) High Stress Hormones linked to heart disease and high blood pressure. Post hoc analyses also found that males were overrepresented on the High Blood Pressure (61.2%), Metabolic Syndrome (63.2%), High Stress Hormones (66.4%) and High Blood Sugars (57.1%) profiles, while females were overrepresented on Healthy (81.9%), Low Stress Hormones (66.3%) and Low Stress Antagonists (95.4%). We also found that, in terms of biomarkers, very low levels of DHEAS reflect a possible 'gender difference' in androgen production associated with stress-related disorders. Overall, these exploratory results strongly advance Carlson and Chamberlain (2005) argument that, to more

effectively address health differences, an allostatic view of health, grounded in a complex systems perspective, is highly useful.

In a paper derived directly from the original STRIVE study where 46 ROTC and civilian students were tested with the first five STRIVE modules while undergoing an EKG, the PhD candidate Jay Wellman, utilized power spectrum analysis of Heart Rate Variability (HRV) for proxy study of emotional regulation by measuring autonomic nervous function. Virtual reality (VR) has become an increasing popular means of administering exposure treatments for anxiety disorders due to the added safety and ability to exert more control over the environment. To date, few studies have attempted to use HRV as an outcome measure in VR research. The ability to induce stress in a virtual environment has been well established, however, the corresponding change in HRV has not. The present study investigated whether the stress induced in VR creates expected changes in HRV. Additionally, the effectiveness of mentor training in increasing variability was investigated. Thirty-nine participants, including psychiatrically normal military cadets from navy/marine and army programs who had never been deployed as well as civilians, had fully usable data for analysis. The combat scenarios demonstrated significant difference in expected directions from baseline using paired-samples *t*-tests. Overall, results support the use of HRV in VR interventions. Suggestions for improvement of mentor scenarios and directions for future research are included.

A study on the California National Guard by the PhD student Jared Rensberger reported highly significant associations between a measure of spirituality and between the ratio between both dehydroepiandrosterone (DHEA) and cortisol and between DHEA-Sulfate (DHEA-S) and cortisol. While cortisol and DHEA are both released concurrently in response to extreme stress and have different effects on the body, it is difficult to interpret their impact separate from one another. This is largely due to the change in hormonal levels across the lifespan. The production of DHEA declines as the person ages, a process known as adrenopause, but cortisol remains stable across the lifespan. Yehuda et al. (2006) propose that considering the ratio of DHEA to cortisol may be a helpful method of determining the role these hormones play in the context of stress and trauma. A higher DHEA-to-cortisol ratio is associated with improved performance in military survival training and fewer dissociative symptoms following stress. Similarly, higher DHEA levels have been shown to be associated with PTSD symptom improvement. Higher DHEA-to-cortisol ratios may therefore indicate higher resilience to stress. While this study did not find significant associations between measures of resilience and the ratio of DHEA to cortisol, the strong effect seen between spirituality and this ratio furthers suggestions between aspects of well-being and stress management.

A fourth paper is being completed by the PhD candidate, Eric An, who is evaluating HRV among the National Guard soldiers. This paper will be completed prior to his graduation in May 2016, and will both confirm and extend the findings reported by Wellman.

Episode 5: *Loss of a Leader*

This episode, developed under funding from this ONR project, can be viewed at:

http://youtu.be/kQS0iK7JYOo

Publications

The four papers generated from this project are attached to this report.

Allostatic Load and Health Risk Outcomes: A Complex Systems Approach

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Allostatic Load and Health Risk Outcomes:
A Complex Systems Approach

ABSTRACT

Using data (N=1151) from the *MIDUS II* study, we advanced the measurement of allostatic load (AL) by modeling it as a complex system, based on the tools of case-based computational modeling. More specifically: (1) we used factor analysis to arrive at a 7-factor solution for the underlying structure of AL; (2) used a topographical neural net and k-means cluster analysis to create a catalogue of nine key AL profiles; and (3) regressed these profiles on 23 health outcomes to construct a health-risk typology for each. In terms of highlights: (a) our *Healthy* profile had few clinical problems, revealing a unique and positive view of biomarkers and health risk factors in combination; (b) *Pro-Inflammatory* 59% with high blood pressure and 36% diabetes; (c) *Low Stress Hormones* linked to heart disease, TIA/Stroke, diabetes and circulation problems; and (d) *High Stress Hormones* linked to heart disease and high blood pressure. Post hoc analyses also found that males were overrepresented on the *High Blood Pressure* (61.2%), *Metabolic Syndrome* (63.2%), *High Stress Hormones* (66.4%) and *High Blood Sugars* (57.1%) profiles, while females were overrepresented on *Healthy* (81.9%), *Low Stress Hormones* (66.3%) and *Low Stress Antagonists* (95.4%). We also found that, in terms of biomarkers, very low levels of DHEAS reflect a possible 'gender difference' in androgen production associated with stress-related disorders. Overall, these exploratory results strongly advance Carlson and Chamberlain (2005) argument that, to more effectively address health disparities, an allostatic view of health, grounded in a complex systems perspective, is highly useful.

KEY WORDS: allostatic load, complexity theory, health risk outcomes, health disparities, computational modeling, case-based modeling.

Allostatic Load and Health Risk Outcomes:
A Complex Systems Approach

1. Allostatic Load as a Complex System

In 2005, Carlson and Chamberlain published "Allostatic Load and Health Disparities: A Theoretical Orientation," in *Research in Nursing & Health*, arguing that, if we are to more effectively address "racial and ethnic health disparities," a change in thinking is necessary. Healthcare providers and researchers (from nurses and biologists to physicians and epidemiologists) need to adopt a more *complex systems* view of patients – both in terms of (a) the complex network of bio-physiological systems of which patients are comprised; and (b) the wider network of socio-ecological systems and communities in which they reside. More specifically, they need to understand the intersection of these two complex systems – the socio-ecological and the bio-physiological – to make better sense of health outcomes; that is, "how the social environment exerts a cumulative impact on the physical and mental well being of individuals" (2005, p. 308).

For Carlson and Chamberlain – and for a growing network of likeminded researchers – the concept that best embodies this necessary "change in thinking" is *allostatic load.* Allostatic load (AL) is a highly useful theoretical framework – introduced by McEwen and colleagues (e.g., McEwen, 2000; McEwen & Seeman, 1999; McEwen & Stellar, 1993) – for understanding the cumulative health costs ("wear and tear") associated with stress, particularly short-term-intense or chronic distress.

As Figure 1 shows, the theoretical framework for AL follows a complex trajectory: situated within a wider set of intersecting socio-ecological systems (Model B), an individual's perceived distress (i.e., environment, life events, trauma) causes many of

the body's key allostatic systems – a complex, nonlinear network of interactive and adaptive mediators (e.g., blood pressure, cardiovascular, metabolic, etc) – to shift into a state of relative disequilibrium to maintain wellbeing (Juster et al., 2010, p. 3). Often times, however, particularly when distress is short-term-intense or chronic, this sustained disequilibrium leads to dysregulation (Model A), which causes significant dysfunction/ damage to these allostatic systems; which, in turn, leads to significant negative health outcomes (e.g., heart disease, cancer, depression, alcoholism, PTSD) (For a complete theoretical overview, see McEwen, et al., 2012).

Given its theoretical complexity, AL has shown also great potential as an interdisciplinary tool for assessing *cumulative health risk outcomes* (McEwen et al., 2012). As Gallo et al (2014) state, "[T]he allostatic load framework provides an integrative approach that may better characterize the *cumulative* impact of dynamic and nonlinear influences across major biological regulatory systems." In this way, AL links to a variety of fields focused on the negative impact that stress has on health and wellbeing; particularly across the life-course and across key antecedent socio-ecological factors such as gender, residence, ethnicity, trauma and – a current major focus – *health disparities* (e.g., Castellani et al., 2014; Mair et al., 2011; Merkin et al., 2014).

2. The Challenge of Measuring Allostatic Load

Given its potential, researchers have developed a variety of ways to measure AL (Juster et al., 2010). The challenge, however, is built into the very nature of what makes AL unique. As Carlson and Chamberlain argue (2005), AL is an emergent, self-organizing, nonlinear, dynamic, evolving, longitudinal network of intersecting allostatic systems

situated within a wider web of intersecting socio-ecological systems (Figure 1). This complexity has created five major challenges:

First, there is the issue of what *biomarker panel* best operationalizes the complexity of AL (Juster et al., 2010). As Gallo *et al.* (2014) point out, "Allostatic load is typically operationalized as a composite of biological markers representing multiple systems, especially the neuroendocrine, cardiovascular, metabolic, and immune systems" (p.479). However, at present, variability in panels is significant (McEwen et al., 2012).

Second, the causal pathways amongst the allostatic systems for these panels remain under-theorized (Merkin et al., 2014; Schulkin, 2003; Sterling, 2004). For example, waist-to-hip ratio has been related to (a) elevated heart rate and blood pressure, (b) dysregulated-HPA axis activity, (c) decreased high-density lipoprotein (HDL) cholesterol, and (d) high glucose, insulin, and triglyceride levels (Ljung et al., 2000; Wing et al., 1991).

Third, AL is measured as a composite index; that is, a single score. In addition to the restricted predictive value of this approach (Geronimus et al., 2015; McEwen et al., 2012), dichotomizing biomarkers to achieve a composite score (as is often done) dilutes variability. Also, any sort of "summing" of biomarkers, based on dichotomization, potentially gives equal weight to all markers. And, finally, reducing AL to a single composite negates the ability to evaluate causal patterns amongst the biomarkers (Gersten, 2008; Hawkley et al., 2011; Loucks et al., 2008).

Fourth, there is the issue of how AL is differentially expressed in groups as a function of (1) differences in antecedent socio-ecological factors and (2) differences in antecedent bio-allostatic makeup (Gallo et al., 2014; McEwen et al., 2012). In other

words, there is 'one' definitive causal pathways model for AL; instead, there are multiple causal pathways, expressed in the form of multiple causal outcomes, both in terms of (1) different AL profiles and their different associated typologies of health risk outcomes.

Fifth, there is the issue of dynamics and time. The majority of studies, to date, are cross-sectional. More longitudinal research is therefore necessary, examining AL for different sub-populations and cohorts, as well as how it evolves across time (Gallo et al., 2014; Juster et al., 2010).

3. Purpose of Current Study

Hence we come to the purpose of the current study: *we seek to advance the measurement of allostatic load by modeling it as a complex system, based on the methodological tools of case-based computational modeling*. The complexity sciences constitute an across-the-academy field of study, focused on rethinking scientific inquiry from a *complex systems* perspective (Capra & Luisi, 2014). Much of this rethinking is focused on method, particularly computational modeling, which uses high-powered computers and brute-force algorithms to arrive at 'approximate' models for highly complex data (Mitchell, 2009). Examples include genetic algorithms, agent-based modeling, networks, and more recently, case-based computational modeling (Byrne & Callaghan, 2014).

For those new to case-based computational modeling (CBM), several quick comments are necessary. While statistics generally focuses on the average, aggregate behavior of samples, CBM focuses on cases, specifically how they aggregate and cluster into similar groups (Castellani & Rajaram, 2012). The strength of CBM is its ability to: (1) identify sub-group differences (*profiles*) amongst highly complex data and (2) explore

the *different* causal pathways of these profiles, based on differences in key variables (Byrne & Callaghan, 2014; Byrne & Ragin, 2009). Given its utility, CBM is used rather widely (Byrne & Ragin, 2009). In all such instances, cases and their variable-based profiles is the focus (Castellani and Rajaram, 2012).

We employed CBM for the current study to facilitate three advances in the measurement of AL as a complex system. (For more details, see Methods):

Advance 1: First, we used CBM to develop a multi-system, factor analytic measure of AL; which, we argue, has the following measurement advantages: it allows researchers to (a) bypass simplistic indices of AL; (b) suggest a possible 'gold standard' biomarker panel; (c) engage in a theoretical exploration of different causal pathways amongst key AL mediators; and (d) preserve the multi-system complexity of AL while (simultaneously) decomposing it onto a meaningful set of factors.

In terms of establishing a gold standard, we followed the theoretical framework of McEwen *et al.* (2012) and Seeman *et al.* (2010b), focusing on twenty key components of major allostatic systems (See Methods). Nonetheless, we do acknowledge Gallo *et al.'s* (2014) important caveat that, while a gold standard is useful, variance in biomarker panels seems to have only "a modest bearing" on the "predictive utility" of AL (p. 479).

As a final point, our approach, while novel, has precedent in the AL literature (Hawkley et al., 2011; McEwen et al., 2012; Seeman et al., 2010b) and it is based on preliminary research: when compared to a composite index measure of AL using linear regression, our preliminary factor solution predicted significant variance in depression, anxiety, and medical outcomes (Buckwalter et al., 2011). Seeman *et al.* (2014) also successfully used our preliminary solution to examine health disparities based on social

status, demonstrating its potential as a clinical tool. In terms of the current study, we examined a larger sample to explore further our preliminary factor solution (See Table 1).

Advance 2: Second, we used CBM to construct a catalogue of AL profiles (See Table 3 and Methods for details), based on subject scores from our factor analytic solution (See Table 1). In terms of advancing measurement, this catalogue has two advantages: it allows researchers to explore how AL manifests itself differently in people as a function of (a) differences in their antecedent bio-allostatic makeup and (b) differences in their antecedent socio-ecological factors. As proof of concept, in terms of the former, we will (a) explore the factor analytic structure for the AL profiles in our catalogue and (b) conduct a post hoc analysis of the role *gender* plays in our results.

Advance 3: Finally, we used CBM to create a health risk outcomes typology for each AL profile. For our study, we hypothesize that our AL profiles, once identified, will reflect group differences in overall biological health or conversely dysregulation. In terms of advancing measurement, our health risk outcomes typologies will allow researchers to link AL to the short or long-term health of groups, particularly in terms of health disparities.

Three Caveats: Before we proceed, however, three caveats are needed. First, the exploratory nature of our study needs to be acknowledged. Second, given the limitations of any one study, we did not address all five measurement issues. For example, while more longitudinal research is necessary, our study is based on a cross-sectional, middle-aged sample (Mean Age = 55.0; SD = 11.8) (See Methods). Third, while some may argue for exploring the factor analytic structure of AL based on gender or ethnicity, current research suggests otherwise. As Seeman *et al.* (2010b) recently concluded: "We

sought to test a hypothesized metafactor model of allostatic load composed of a number of biological system factors, and to investigate model invariance across sex and ethnicity.... A 'metafactor' model of AL as an aggregate measure of six underlying latent biological subfactors was found to fit the data.... There was little evidence of model variance across sex and/or ethnicity" (p. 463).

METHODS

Subjects:

For this study we utilized archival data from the *Midlife Development in the United States (MIDUS)* study (Deinberg et al., 2010), a national survey by the MacArthur Midlife Research Network in 1994/95, which included data from over 7,000 Americans aged 25 to 74. The purpose of the survey was broad, investigating the role of behavioral, psychological, and social variables on a variety of health outcome measures. In 2002, the University of Wisconsin-Madison carried out a longitudinal follow-up of the original MIDUS respondents. This second initiative (MIDUS II) (Deinberg et al., 2010) had five research foci, one of which included comprehensive biomarker assessments obtained from a subsample of MIDUS respondents. For the current study, the number of subjects with valid biomarker data used was 1151. Missing data from this total were removed pairwise rather than using a substitution. A total of 4.8% of data were missing when calculating the final clusters/types for our AL catalogue. In terms of overall demographics, our sample was 57% female (N=656) with a mean age of 55.0 (SD = 11.8). Also, our sample was representative of the original MIDUS sample. As a final point, because we did not construct an index, we did not explore medication data.

However, for those interested in how medication data can be used to augment our preliminary factor solution, see Seeman *et al.* (2014).

AL Biomarker Panel:

We used 20 MIDUS II biomarkers – nearly double the average used in 58 studies reviewed by Juster et al. (2010). For details on these biomarkers, see Friedman et al. (2009) and Love et al. (2010). We also made sure that all 20 biomarkers were utilized in at least two published studies reviewed by Juster et al.'s (2010). In terms of theory, we organized our biomarkers into five physiological systems. **(1)** Biomarkers from the *neuroendocrine* system included (a) three catecholamines (norepinephrine, epinephrine, and dopamine); (b) the androgen dehydroepiandrosterone sulfate (DHEA-S); and (c) the glucocorticoid cortisol – all of which are involved in the body's stress reaction. **(2)** The *cardiovascular/respiratory* system biomarkers were systolic and diastolic blood pressure and peak expiratory flow (which is the maximum speed of expiration and an indicator of airflow through the bronchi). **(3)** The *metabolic* system biomarkers are well-known indicators of cardiovascular health. This study included total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, hemoglobin Alc (HbAlc), and insulin. **(4)** We also included biomarkers from the *immune* system, including (a) C-reactive protein (CRP), an acute phase reaction protein that promotes inflammation; (b) interleukin-6 (IL-6), a mediator of the acute phase response that acts as a pro-inflammatory element; (c) the anti-inflammatory cytokine, fibrinogen, which functions as a blood clotting factor that promotes coagulation but increases risk of thrombosis when excessive, and (d) the insulin-like growth factor (IGF-1), a protein that

mediates the effects of growth hormone and inhibits cellular apoptosis. **(5)** Finally, a biomarker of *anthropometric* status, which measures body habitus, waist-to-hip ratio (WHR) was used.

Health Risk Outcomes Measures:

For our study we used several MIDUS-II self-report health outcomes: heart disease, high blood pressure, circulation problems, blood clots, heart murmur, stroke, anemia, diabetes, cholesterol problems, asthma, tuberculosis, emphysema/COPD, thyroid disease, peptic ulcers, cancer, colon polyps, arthritis, glaucoma, cirrhosis, alcoholism, depression, and blood transfusion before 1993. (For details, see Friedman et al., 2009; Love et al., 2010).

Statistical/Computational Analyses:

As discussed in the introduction, to model AL as a complex system we employed a case-based modeling approach (Byrne & Ragin, 2009), specifically, the *SACS Toolkit* (Castellani & Rajaram, 2012; Rajaram & Castellani, 2012, 2014). The SACS Toolkit is a computationally grounded, mixed methods platform for modeling complex systems as sets of cases. While the SACS Toolkit draws upon a wide variety of computational and statistical techniques, for the current study we used four: the Kohonen self-organizing map (SOM), k-means cluster analysis, principle components analysis (PCA), and logistic regression. While most readers should be familiar with logistic regression, the SOM, PCA, and k-means are less commonly known. As such, we provide further detail here on our usage of them, which involved the following two steps:

Step 1: Factor Analytic Solution: We began with PCA (a type of factor analysis) in order to: (1) identify the major and multiple pathways/relationships amongst our

twenty biomarkers, based on their factor loadings; and (2) name the factors in the PCA solution. For those new to PCA, see Table 1, which shows the factors we found, their names, and the loadings (scores) for each of the 20 biomarkers on each factor.

Prior to PCA, we used a *parallel analysis* to identify the appropriate number of factors to be extracted. For more on this technique, see Lautenschlager (1989). Also, given that our factors constitute highly complex and interdependent AL biological systems, we did not assume that they would be independent. Instead, given our complex systems approach, we treated their causal pathways as complex, multiple, self-organizing, emergent and nonlinear. As such, we used a promax (oblique) rotation.

The resulting factor structure was interpreted by requiring a loading of .60 to retain each biomarker for each factor identified. All biomarkers were retained on the factor for which they loaded the highest. No item loaded over .60 on more than one factor and all items loaded on at least one factor. Factors were named based on the allostatic systems the biomarkers represented – note, an allostatic system could be represented by more than one factor; or, conversely, a factor could represent more than one system.

Finally, after the PCA was completed, all biomarkers that loaded saliently on a factor were used to form standardized scores. This n-dimensional vector profile for each case in the database was then used, in turn, to complete Step 2.

Step 2: Constructing our AL Catalogue: With our factors identified, we sought to assemble a catalogue of AL profiles. To do so, we employed the SOM and k-means cluster analysis (Byrne & Uprichard, 2012; Castellani & Rajaram, 2012). For those new to these techniques, k-means and the SOM cluster cases by searching for a simpler set of key profiles, with cases similar to these profiles positioned near them, based on some

neighborhood (similarity) function (Jain, 2010; Kuo et al., 2002a, 2002b). The SOM is particularly useful because it displays its solution as a map – See Figure 2.

For those more familiar with these techniques, we provide the following detail: k-means is an iterative, partitional (as opposed to hierarchical) clustering technique that seeks a single clustering solution for some proximity matrix. For k-means, reference vectors are centroids, representing the average for all the cases in a cluster. The SOM is a topographical neural net that maps high-dimensional data onto a smaller, three-dimensional space, while preserving, as much as possible, the complex patterns of relationships amongst these data. For the SOM, reference vectors are actual points (neurons), which represent the weighted average of the AL cases clustering around it. These methods were used in combination as follows:

K-means was used first because it requires that the number of clusters be identified ahead of time, based largely on some rationale (usually theoretical or previous empirical research), even if tentative or conjectural. Following convention, we ran our k-means with normalized data (as mentioned at the end of Step 1), using a Euclidian measure of distance, with the convergence criterion set to zero. After several runs, all outliers and cases with only partial data were removed. An ANOVA table with unstandardized F statistics was also generated to determine the relative impact each component in the AL profile had on the final cluster solution.

To construct our AL profiles, we evaluated the centroids (clusters) shown in Table 2. Also, to help determine the crispness of the clusters, box plots were examined for outliers. The distance measures for all cases relative to their clusters were also normalized as z-scores and the standard deviation for each cluster computed.

Next, the SOM was run. Because the SOM is unsupervised, if it arrives at a solution similar to the k-means it provides effective corroboration. Analyses were conducted using the SOM Toolbox, a freeware package for MATLAB (Kohonen et al., 2010). The SOM graphs its cluster solution onto a variety of three-dimensional maps. For the current study, as shown in Figure 2, we used the u-matrix and components map. On the u-matrix, cases most like one another are graphically positioned as nearby neighbors, with the most unlike cases placed furthest apart. The u-matrix and components map are also topographical: valleys (darker colored) areas are more similar in AL profile; while hilly, brighter colored areas are more distinct. The component maps visualize how each of the factors from our factor solution contributed to the making of a profile and to the positioning of cases on the u-matrix.

RESULTS AND DISCUSSION

To advance the measurement of AL and, in turn, its theoretical understanding as a complex system, we did the following: (1) used PCA to determine the underlying factor analytic structure of AL; (2) used the SOM and k-means to construct a catalogue of AL profiles; and (3) regressed the resulting AL profiles on a series of health outcomes to construct a health-risk typology for AL. Our results are as follows:

Factor Solution:

As shown in Table 1, our PCA arrived at a 7-factor solution, accounting for 72.3% of the total variance. Furthermore, despite the complex causal pathways amongst the twenty biomarkers, the factor loadings were, overall, very clear. The only exceptions were: (a)

Waist-to-Hip on Blood Pressure, Blood Sugars and Stress Antagonists; (b) *Insulin* on Pro-Inflammatory Elements and Blood Sugar; (c) *Triglycerides* on Blood Pressure; (d) *Glucose* on Metabolic Syndrome; and (e) *Peak Flow* on Metabolic Syndrome. Still, these additional loading were only .411 or lower. Based on the factor loadings, the seven factors were named as follows: (1) Blood Pressure, (2) Metabolic Syndrome, (3) Cholesterol, (4) Pro-Inflammatory Elements, (5) Stress Hormones, (6) Blood Sugars and (7) Stress Antagonists.

Overall, then – and, as we will demonstrate in the remainder of this section – our results support the usage of factor analysis as a robust empirical and biologically plausible solution for the complex latent structure of AL, as argued by Carlson and Chamberlain (2005) and others (Hawkley et al., 2011; McEwen et al., 2012; Seeman et al., 2010b). More specifically, our results suggest that our 20-biomarker panel – given its theoretical grounding (see Introduction and Methods) – may, indeed, serve as a *gold standard* for measuring AL. Finally, the 'goodness of fit' of our PCA solution provides initial support (but by no means confirms) for measuring AL a complex multi-system biological construct, rather than as a simple composite index.

Cluster/Profile Solution:

As shown in Table 2 and Figure 2, the SOM and k-means settled on a catalogue of nine profiles, which we named as follows. *1. Low Cholesterol:* The key feature of this profile is its very low centroid score on Cholesterol (-1.12); and, in turn, Stress Hormones (-.79). The SOM supported these results. However, looking at Map B, the SOM grouped the *Low Cholesterol* (forest green) cases (Profile 2) into three possible sub-groups. Looking

at Map C and Map B together, the top left sub-group has the lowest Cholesterol scores, while the other two sub-groups have the lowest Stress Hormones scores (Map C).

2. Healthy: The centroid scores for this profile were very low on Metabolic Syndrome (-1.08), Pro-Inflammatory Elements (-1.19) and Blood Pressure (-1.10). The SOM supported these results: looking at Map B, the SOM positioned the *Healthy* cases (Profile 2) at the top, colored in light yellow. Looking at this same area for each of the seven factors in Map C (components map), one finds low to very low scores (dark blue) on Blood Pressure, Metabolic Syndrome, Pro-Inflammatory Elements, and Blood Sugar. The only elevated factor on the k-means and SOM was Stress Antagonists, with some cases in Map C scoring high (red).

3. High Blood Pressure: The key feature of this profile is a high score on Blood Pressure (.94) and elevated Stress Antagonists; but, conversely, a low score on Pro-Inflammatory Elements (-.71). In addition, looking at Map B, the SOM grouped *High Blood Pressure* (light blue) into two possible sub-groups – one large, one small – with cases on the left having higher Cholesterol (Map C) and Stress Antagonists (Map C).

4. Low Stress Hormones: This profile (the largest, N=169) had the lowest score on Stress Hormones (-.92) and a high Cholesterol score (.73). Looking at Map B, the SOM supported these results, placing the majority of cases in Profile 4 toward the lower center (light purple), where some of the highest Cholesterol and lowest Stress Hormones scores are found (Map C). But, the SOM also identified a possible (albeit small) subset of cases toward the upper right of Map B, which is lower on Stress Antagonists.

5. Metabolic Syndrome: This profile has the highest score on Metabolic Syndrome (1.22) and high scores on Cholesterol (.73) and Pro-Inflammatory Elements

(.99). The SOM supported these results (Map C), placing *Metabolic Syndrome* cases (Profile 5) at the bottom center-left (melon) of Map B. The SOM also identified a possible small subset of cases in the lower right, very high on Metabolic Syndrome and Pro-Inflammatory Elements, but not as high on Cholesterol.

6. High Blood Sugar: This profile – the smallest (N=35) – has a high Metabolic Syndrome score and the highest Blood Sugars score (3.71); placing the centroid scores for Profile 6 three standard deviations above the mean. The SOM strongly supports these results (Map C), placing Profile 6 (purple) in the lower right corner on Map B.

7. Low Stress Antagonists: This profile has the lowest score on Stress Antagonists (-1.70) and also low scores on Metabolic Syndrome (-.74) and Stress Hormones (.99). The SOM supported these results (See Map C), placing these cases (Profile 7) along the upper right (pink) of Map B. However, the SOM spreads this profile out a bit, overlapping it with *Low Stress Hormone*s, possibly due to their mutual low scores on Metabolic Syndrome and mild scores on Stress Antagonists.

8. High Stress Hormones: Directly opposite of the *Low Stress Hormones* type, this profile (the second largest, N=146) has the highest score on Stress Hormones (1.03) and one of the lowest scores on Cholesterol (-.69). Looking at Map B, however, the SOM did not entirely support these results, breaking Profile 8 (mocha) into two possible groups. The upper-left profile is similar to Table 2; however, the bottom-left profile differs, with high scores on Pro-Inflammatory Elements, Metabolic and Blood Pressure.

9. High Pro-Inflammatory Elements: This profile had the highest centroid score on Pro-Inflammatory Elements (1.08); a high score on Metabolic Syndrome (.73); and, it is important to note, a low score on Stress Antagonists (-.73) and cholesterol (-.82). The

SOM supports these results (grey profile on Map B), although Pro-Inflammatory Elements, Metabolic and Cholesterol do go down as one moves toward the upper-right.

As the results from these nine profiles suggest, a case-based modeling approach further supports AL as a complex system. Furthermore, while our profile catalogue must be considered preliminary, it also demonstrates the possibility of multiple causal pathways and therefore multiple possible models of AL as a complex system.

Health Risk Outcomes Profiles:

As shown in Table 3, using logistic regression, we arrived at a *Health risk outcomes Typology* for each of our *AL Profiles*. To help readers make sense of these results, we created Figure 3, which visually displays the differences between observed and expected frequencies for each self-reported medical condition. In Figure 3, the radii represent all 23 medical conditions. The profiles are circumscribed around these 23 radii based on the results from Table 3. The resulting health risk outcomes profile is shown in red. Scores higher than 0 (the green circle) indicate a greater observed value than expected, whereas scores below 0 indicate a smaller observed value than expected. The most noteworthy scores for each profile are also labeled in red.

Health Risk Outcomes Across Profiles:

Based on entering 'profile membership' as the predictor variable, two key findings emerged. First, across all nine profiles, the most significant health outcomes were heart disease ($p < .001$), high blood pressure ($p < .001$), circulation problems ($p = .003$), blood clots ($p < .022$), anemia ($p < .001$), cholesterol ($p < .001$), diabetes ($p < .001$), peptic ulcers ($p = .048$), cancer ($p = .017$), colon polyp ($p = .005$), arthritis ($p < .001$), blood transfusion before 1993 ($p < .001$). There was, however, no significant predictive effect for profile membership in terms of self-reported heart murmur, TIA/stroke, asthma, COPD, tuberculosis, thyroid, glaucoma, cirrhosis of the liver, alcoholism, or depression. Second, given the underlying assumption that our catalogue of profiles would reflect groups formed by biological dysregulation, the medical conditions most susceptible to AL turned out to show the greatest fluctuations across profiles. In that context we find that cholesterol, blood pressure, arthritis and diabetes were the most susceptible to fluctuations.

Health risk Outcomes Amongst Profiles:

In addition to our overall findings, there were significant differences in the health outcomes for our nine AL profiles. Our results are as follows:

Healthy Outcomes: To begin, there were three profiles with healthy to moderately healthy outcomes. Of the three, the most obvious was *Healthy*; which, in contrast to the other eight profiles, reported, overall, lower health risk outcomes, including exceptionally lower rates of circulation problems, cholesterol and arthritis; as well as low rates of heart disease, diabetes, TIA/stroke, heart murmur and blood clots. Still, this profile reported slightly greater than expected (or equivalent) rates of cancer,

anemia, emphysema, tuberculosis, thyroid disease, and glaucoma. Perhaps most important, when viewed as an emergent complex system, the *Healthy* profile and its associated risk typology illustrated something new: while many of its biomarkers have long been documented to be risk factors for cardiovascular dysfunction, this is the first time, to our knowledge, they have been shown to group together when evaluating the AL outcomes of stress – illustrating, in this case, a positive outcome. For example, less than 1% of the N=138 cases in this profile reported heart disease.

The second healthy profile is *Low Cholesterol*; which, overall, had a health risk outcomes typology close to expected values on most markers. It did, however, have slightly higher rates on a few outcomes, such as heart disease, alcoholism and depression. And, it certainly did not have the markedly low rates found in the *Healthy* profile.

The least obvious healthy profile was *High Blood Pressure*; which under-reported on key stress-related disorders, including high blood pressure (49/56) and cholesterol (38/66). These findings suggest that blood pressure alone may not initiate the cascade of disorders associated with more pivotal profiles, like Pro-Inflammatory Elements; or more likely, it suggests that patients who report high blood pressure are, by the time of self-report, given the older age of our sample, effectively treated.

Unhealthy: In contrast to the health profiles are the other six; which were, to varying degrees, associated with patterns of poor health outcomes; or, alternatively, high rates on key outcomes. As a first example, when analyzing *Metabolic Syndrome*, we found expected high rates for high cholesterol (83/62) and high blood pressure (61/52). But we also found lower than expected rate for heart disease (7/17).

Another example is *High Pro-Inflammatory Elements*, which reported a high rate of heart disease (27/12), high blood pressure (61/37.5), circulation problems (20/10), diabetes (37/12), cholesterol (68/45), cancer (23/15) and arthritis (56/42). This profile also appears to be the most impacted by AL, with consistent over-reporting of cardiovascular and metabolic disorders. It may be relevant that this profile is also low on cholesterol and stress antagonists. The strong association between high levels of pro-inflammatory elements (IL-6, fibrinogen, CRP) and cardiovascular problems is, however, expected (Hedayat et al., 2010; Koenig et al., 2004; Stec et al., 2000). Also expected is the association with diabetes (Guest et al., 2008; Schmidt et al., 1999).

Low Stress Antagonists showed a consistent pattern of over-reporting stress-related health outcomes with the exception of diabetes (6/12); and a significant number of cases in this profile reported high rates of heart disease (24/13), high blood pressure (51/39), thyroid (22/14), blood transfusions (19/11) and arthritis (65/45) – which, when viewed in total, raises suspicion about the role that stress antagonists play in this profile.

The findings for *Low Stress Hormones* and its counterpart *High Stress Hormones* also support the current literature, which can be summarized as follows: while the short term effects of stress hormones – which, in the current study, includes catecholamine and the HPA steroid cortisol – are positive, it appears that long-term circulating effects are negative in heart disease (Adameova et al., 2009). The fact that *Low Stress Hormone* also under-reported heart disease, while *High Stress Hormones* over-reported heart disease and high blood pressure is also consistent with the literature (Adameova et a., 2009). However, while *Low Stress Hormone* reported high rates of anemia (36/25),

peptic ulcers (16/9) and depression (50/39), it also under-reported TIA/Stroke, diabetes, and circulation problems – which may suggest broader effects.

Finally, there is *Low Stress Antagonists*. The typology for this profile is significant because it points to the utility of a case-based approach: our exploratory results suggest etiological clues for patterns not entirely expected, largely because there has not been extensive research into the associations observed. In terms of what we found: the biomarkers loading this profile were IGF-1, DHEA-S, and peak flow. In turn, rates for heart disease, high blood pressure, thyroid disease, blood transfusions, and arthritis were well above expected outcomes; however, diabetes was less than expected.

In terms of interpreting these results, DHEAS (the most common adrenal steroid in the body) declines dramatically with age. However, while the exact progression of DHEAS during stress is poorly documented, it is elevated by stress in the short term. Furthermore, higher levels of DHEAS during stress are associated with less stress at a later time; although a more common explanation is that the ratio of DHEAS to cortisol is crucial in controlling stress and may provide beneficial behavioral and neurotrophic effects (McEwen et al., 2012). Its effect may be similar in the long-term. Bremner *et al.* (2007) reported adult women with a childhood history of sexual abuse and current PTSD had higher levels of DHEAS recorded across a 24-hour period than control women and women with abuse but no PTSD.

Post Hoc Results: Allostatic Load and Gender

Following Carlson and Chamberlain's (2005) focus on decreasing health disparities (which is the major trend in the current AL literature – See Introduction), we explored,

post hoc, the influence of *gender* on our results (Ellis & Giudice, 2014; Mair et al., 2011; Merkin et al., 2014). Overall, we found that women were overrepresented on *Low Stress Antagonists* (95.4%), *Healthy* (81.9%) and *Low Stress Hormones* (66.3%). In contrast, men were overrepresented on *High Blood Pressure* (61.2%), *Metabolic Syndrome* (63.2%), *High Stress Hormones* (66.4%) and *High Blood Sugars* (57.1%). In contrast, however, the 'percentage female' for *Low Cholesterol* (56.3%) and *High Pro-Inflammatory Elements* (57.7%) was similar to our overall sample (57% female).

The fact that men were overrepresented on *High Stress Hormones*, as well as the profiles characterized by the downstream effects of these hormones, namely *High Blood Pressure* and *Metabolic Syndrome*, leads us to question if men are more susceptible to the negative effects of SAM and HPA hormones. Furthermore, women are overrepresented on the profiles comprising factors consistent with allostasis, namely *Healthy* and *Low Stress Hormone*, while they almost entirely comprise the *Low Stress Antagonists* profile.

The post hoc results for *Low Stress Antagonists* suggests a unique gender-based stress response. The key biomarkers on this profile are peak flow, DHEAS, and IGF-1. While DHEAS is the most common steroid in both men and women, not only having a mild androgenicity effect, it is also the precursor from which all other steroids are metabolized. It drops in a strictly linear fashion with age in both men and women, with women having lower levels than men throughout the lifespan (Berr et al., 1999; Böttner et al., 2004; Fukai et al., 2009; Hernández-Morante et al., 2008). However, this pattern of decline is complicated in old age by the emergence of subgroups that show an increase in levels (after correcting for regression to the mean) for 15% of women and 5% of men (Morsink et al., 2007). The cause of this unexpected finding is unknown and has led

previous studies to conclude that "unknown fundamental gender differences" in adrenal androgen production and excretion might be the cause (Tannenbaum et al., 2004).

In terms of the current study, however, while we cannot *directly tie* our post hoc results to the gender differences described in the current literature, our results also suggest that very low levels of DHEAS may reflect some "unknown fundamental gender differences" in androgen production, which is seemingly associated with the emergence of stress-related disorders. IGF-1 is secreted by the liver and is important for both the regulation of normal physiology and a number of pathological conditions, most notably cancer. While its role in stress is poorly understood, it is reported to be lower in patients suffering multiple traumas (Jeevanandam et al., 1992); and few would argue that it does not play a relevant role in stress. Reviews of the studies on DHEA supplementation during the 1990's (and even more recently) find reports of a rise in IGF-1 subsequent to DHEA administration (Papierska et al., 2012). Interestingly, IGF-1 is also reported to rise in association with cortisol. In apparent contradiction, however, several epidemiological studies have found that low IGF-1 is a risk factor for metabolic syndrome. It is difficult to know what biological stress-related functions are driving the high level of self-reported medical difficulties. Also, it is not clear how knowing they are almost exclusively women is informative, yet this profile may well wave a red flag for further research into this dimension of allostatic load, a reasonable outcome of our exploratory study.

Limitations of Current Study:

Given the exploratory nature of the current study, several limitations are important to highlight – for more, see Introduction and Methods. First, the health risk outcomes for our study were self-report. Second, we did not use medication data. Third, our study was cross-sectional. It is therefore necessary for future research to confirm our results with non-self-report and medication data, as well as a employ longitudinal or cohort design, particularly in order to examine how our factor structure, AL profiles, and health risk outcomes typology change as a function of time or key antecedent socio-ecological factors, including medical treatment, health behaviors, change in residence, and so forth.

CONCLUSIONS

Over the last several decades, the complexity sciences have sought to demonstrate the utility of thinking about health problems (particularly health disparities) in holistic, complex systems terms (Byrne & Callaghan 2014). Allostatic load constitutes one such advance. However, the measurement of this concept, as a complex system, had yet to be fully developed – hence the call made by Carlson and Chamberlain (2005). In response, the current study sought to make one small advance, in order to demonstrate, in exploratory fashion, the utility of modeling and measuring allostatic load as a complex system, including its links with one antecedent socio-ecological factor, namely gender. More specifically, we sought to demonstrate the multiple causal pathways within AL – which we defined as case-based profiles – and their links with different typologies of health risk outcomes. These profiles and health risk outcomes typologies also make one small step toward indices that can be used by nurses and clinicians, as well as biologists and epidemiologists to more effectively understand and treat the different and cumulative health costs associated with stress.

Table 1. Allostatic Load Seven Factor Structure Solution

	Factors/Components*						
	Blood Pressure	**Metabolic Syndrome**	**Cholesterol**	**Pro-Inflammatory Elements**	**Stress Hormones**	**Blood Sugars**	**Stress Antagonists**
Biomarkers							
Systolic BP[a]	**.880**	.158	.060	.132	.054	.130	-.106
Diastolic BP[a]	**.883**	.181	.120	-.052	.141	.020	.220
Waist to hip ratio	.305	**.700**	-.090	.113	.150	.308	.294
HDL[b]	-.096	**-.829**	.103	-.084	-.191	-.129	-.122
Insulin	.082	**.677**	.030	.379	.025	.411	-.007
Triglycerides	.164	**.786**	.297	.113	.039	.235	-.093
Total cholesterol	.099	-.005	**.980**	.021	-.033	.011	-.011
LDL[c]	.098	.095	**.935**	.021	.040	-.077	.093
IL6[d]	.030	.271	-.141	**.786**	.000	.169	-.257
Fibrinogen	.001	-.009	.092	**.804**	-.037	.148	-.096
C Reactive Proteins	.071	.249	.100	**.816**	.033	.185	-.259
Cortisol	.094	-.046	-.008	-.119	**.613**	-.093	.264
Norepinephrine	.124	.237	.006	.124	**.889**	.075	-.001
Epinephrine	.112	.077	-.028	-.085	**.855**	-.016	.178
Dopamine	.044	.190	.000	.020	**.888**	-.006	.124
Hemoglobin A1c	.036	.208	-.059	.238	-.018	**.887**	-.163
Glucose	.115	.355	-.015	.130	.006	**.895**	-.015
DHEAS[e]	-.005	.127	.110	-.098	.226	-.005	**.729**
Peak Flow	.208	.307	-.089	-.286	.111	-.004	**.629**
IGF-1[f]	.031	-.081	.020	-.190	.026	-.162	**.719**

* The allostatic load factor structure was obtained using a principal components analysis with promax solution. Biomarkers were retained for the factor on which they loaded the highest, with a minimum loading of .613.

[a] Blood pressure

[b] High density lipoprotein

[c] Low density lipoprotein

[d] Interleukin 6

[e] Dehydroepiandrosterone sulfate

[f] Insulin-like growth factor

Clusters[a]

Factor/Components[b] Range (min – max)	1 Low Cholesterol	2 Healthy	3 High Blood Pressure	4 Low Stress Hormones	5 Metabolic Syndrome	6 High Blood Sugars	7 Low Stress Antagonist	8 High Stress Hormones	9 High Pro-Inflammatory Elements	ANOVA F-test[d]
Stress Hormones (-3.02 – 3.11)	-.79[c]	.33	.35	-.92	.66	-.22	-.62	1.03	-.30	118.41*
Metabolic Syndrome (-2.81 – 2.90)	-.55	-1.08	-.40	.16	1.22	1.00	-.74	12	.95	**177.97***
Pro-Inflammatory (-3.03 – 3.08)	-.41	-1.19	-.71	.29	.99	.57	-.27	.12	1.08	**154.72***
Cholesterol (-4.69 – 2.75)	-1.12	.06	.42	.73	.73	-.01	-.08	-.69	-.82	93.77
Blood Sugars (-1.83 – 6.70)	-.32	-.48	-.36	-.13	.18	3.71	-.25	.08	.36	**215.42***
Stress Antagonists (-3.86 – 2.26)	.31	.22	.58	.14	.35	-.10	-1.7	.30	-.73	102.06*
Blood Pressure (-3.91 – 3.17)	-.60	-1.10	.94	-.06	.47	.26	.15	.21	-.52	80.78*
	N=96[e]	N=138	N=155	N=169	N=144	N=35	N=109	N=146	N=104	

[a] This 9-cluster solution was obtained using k-means, with standard Euclidian distance measures; convergence criterion was set to zero.
[b] These are the seven factors from Table I, used to construct the different profiles for the nine clusters. Included below each factor is its min and max score possible, which comes from summing the biomarkers that loaded on it and converting this sum into a z-score.
[c] The score for each case, for each of the seven factors, was computed (as noted in ***b*** above) by summing each case's scores on the biomarkers for each factor, as shown in Table I. In turn, these summed factor scores were converted into z-scores to normalize the data.
[d] Unstandardized F scores (ANOVA) demonstrating, for descriptive purposes only, the relative impact the seven factors had in determining cluster membership (* = F test was significant at .000. The factors with the three highest scores are highlighted).
[e] Number of cases in each cluster.

Table 3. Allostatic Load Clusters and Their Differences on Key Self-Reported Health Measures

		Cluster[a]																	
		1 Low Cholesterol		2 Healthy		3 High BP		4 Low Stress Hormone		5 Metabolic Syndrome		6 Hi Blood Sugars		7 Low Stress Antagonist		8 High Stress Hormone		9 High Pro-Inflammatory	
Health Assessment[b]		**Yes**	**No**	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**
Heart Disease	Observed[c]	15	81	1	137	10	145	12	157	7	136	8	26	24	85	26	119	27	75
	Expected	11.40	84.60	16.40	121.60	18.50	136.50	20.10	148.90	17	126.00	4.10	29.90	13.00	96.00	17.30	127.70	12.20	89.80
High Blood Pressure	Observed	29	63	8	129	49	102	58	110	61	81	15	20	51	55	63	82	61	42
	Expected	33.89	58.68	49.92	86.45	55.75	96.54	61.22	106.01	52.47	90.86	12.75	22.08	38.63	66.89	52.84	91.49	37.53	64.99
Circulation Problems	Observed	9	86	9	128	12	139	10	157	16	128	7	28	15	93	11	130	20	78
	Expected	9.61	85.30	13.87	123.01	15.38	136.48	16.90	149.94	14.57	129.29	3.54	31.43	10.93	96.97	14.27	126.60	9.92	87.99
Blood Clots	Observed	5	91	4	134	4	151	11	157	5	139	4	31	6	102	9	137	13	91
	Expected	5.35	90.65	7.69	130.31	8.64	146.36	9.37	158.63	8.03	135.97	1.95	33.05	6.02	101.98	8.14	137.86	5.80	98.20
Heart Murmur	Observed	12	83	14	124	17	138	28	139	19	124	4	31	16	90	28	116	18	85
	Expected	13.63	81.28	19.80	118.07	22.24	132.61	24.11	143.74	20.52	122.35	5.02	29.94	15.21	90.69	20.67	123.20	14.78	88.12
TIA/Stroke	Observed	6	90	2	136	6	149	2	165	4	139	2	33	6	101	8	138	7	97
	Expected	3.78	92.13	5.43	132.44	6.10	148.75	6.58	160.27	5.67	138.20	1.38	33.59	4.21	102.69	5.75	140.12	4.10	99.81
Anemia	Observed	17	79	27	110	13	142	36	133	13	130	4	31	26	83	11	135	16	87
	Expected	14.32	81.68	20.43	116.57	23.12	131.88	25.20	143.80	21.33	121.67	5.22	29.78	16.26	92.74	21.77	124.20	15.36	87.64
Cholesterol Problems	Observed	42	53	28	110	38	111	79	87	83	57	19	14	49	57	66	78	68	35
	Expected	41.52	52.95	60.31	76.92	65.99	84.17	72.99	93.09	61.62	78.59	14.86	18.95	46.76	59.64	62.93	80.27	45.01	57.41
Diabetes	Observed	2	93	4	134	6	149	10	159	11	133	28	7	6	103.0	19	126	37	65
	Expected	10.68	84.15	15.52	122.23	17.43	137.29	19.00	149.69	16.19	127.55	3.94	31.00	12.26	96.55	16.41	129.32	11.58	91.23
Asthma	Observed	13	83	11	127	16	139	18	150	23	121	3	31	16	92	14	130	19	85
	Expected	11.69	84.22	16.81	121.07	18.88	135.98	20.58	148.26	17.54	126.33	4.14	29.83	13.15	94.75	17.54	126.33	12.67	91.24
Emphysema/ COPD	Observed	2	93	5	133	1	154	3	165	2	142	1	34	5	103	4	141	8	96
	Expected	2.69	92.22	3.91	133.96	4.40	150.46	4.79	164.05	4.08	139.78	0.99	33.98	3.06	104.84	4.11	140.75	2.95	100.96
Tuberculosis	Observed	1	95	2	136	0	155	0	168	1	143	0	35	1	108	0	146	1	103
	Expected	0.53	95.47	0.76	137.24	0.85	154.15	0.92	167.08	0.79	143.21	0.19	34.81	0.60	108.40	0.80	145.20	0.57	103.43
Positive TB Skin Test	Observed	5	90	8	130	7	146	11	158	6	137	7	28	13	96	18	126	14	90
	Expected	7.76	87.24	11.27	126.73	12.49	140.51	13.80	155.20	11.68	131.32	2.86	32.14	8.90	100.10	11.76	132.24	8.49	95.51
Thyroid Disease	Observed	14	81	17	121	17	138	25	144	14	130	4	31	22	87	10	135	16	88
	Expected	12.19	83.73	17.52	120.36	19.68	135.18	21.45	147.39	18.28	125.59	4.44	30.53	13.84	95.06	18.41	126.46	13.20	90.70
Peptic Ulcer	Observed	2	93	3	133	5	149	16	151	8	135	0	34	8	101	7	138	7	95
	Expected	4.90	90.10	7.01	128.99	7.94	146.06	8.61	158.39	7.37	135.63	1.75	32.25	5.62	103.38	7.48	137.52	5.31	97.69
Cancer	Observed	16	80	22	116	10	144	18	151	21	122	4	31	22	87	21	124	23	81
	Expected	13.79	82.21	19.82	118.18	22.12	131.88	24.28	144.72	20.54	122.46	5.03	29.97	15.66	93.34	20.83	124.17	14.94	89.06
Colon Polyp	Observed	18	77	17	121	19	136	35	133	22	121	8	26	31	78	30	115	28	74
	Expected	18.13	76.78	26.33	111.54	29.58	125.28	32.25	136.60	27.29	115.58	6.49	27.48	20.80	88.10	27.67	117.20	19.46	82.44
Arthritis	Observed	30	61	35	99	59	93	71	94	69	74	17	17	65	38	59	84	56	41
	Expected	39.50	51.50	58.17	75.83	65.98	86.02	71.62	93.38	62.07	80.93	14.76	19.24	44.71	58.29	62.07	80.93	42.11	54.89
Glaucoma	Observed	3	93	5	133	6	148	8	159	2	142	0	35	7	101	4	141	7	97
	Expected	3.69	92.14	5.30	132.44	5.92	147.80	6.46	161.24	5.53	138.20	1.34	33.59	4.15	103.65	5.61	140.12	4.00	99.81
Liver Cirrhosis	Observed	2	94	1	137	3	152	4	165	3	141	0	35	1	107	3	143	4	99
	Expected	1.84	94.16	2.65	135.35	2.98	152.02	3.24	165.76	2.76	141.24	0.67	34.33	2.07	105.93	2.80	143.20	1.98	101.02
Alcoholism	Observed	6	90	3	135	9	145	3	166	2	139	0	35	2	106	4	142	3	101
	Expected	2.82	93.18	4.05	133.95	4.52	149.48	4.96	164.04	4.14	136.86	1.03	33.97	3.17	104.83	4.28	141.72	3.05	100.95
Depression	Observed	24	71	29	105	25	130	50	117	38	105	7	28	30	78	26	118	26	75
	Expected	22.39	72.61	31.58	102.42	36.53	118.47	39.36	127.64	33.70	109.30	8.25	26.75	25.45	82.55	33.94	110.06	23.80	77.20
Blood Transfusion	Observed	7	88	7	128	11	141	20	146	7	134	5	30	19	88	9	136	22	78
	Expected	9.45	85.55	13.42	121.58	15.12	136.88	16.51	149.49	14.02	126.98	3.48	31.52	10.64	96.36	14.42	130.58	9.94	90.06

[a] Clusters from the 9-cluster solution shown in Table II. [b] Health Assessments used in Study—see Methods section for more details.

[c] Logistic regression was used to test the overall chi-square obtained when testing membership in all clusters. The current table was created for visual inspection of obvious patterns in terms of comparing observed frequency to expected frequency across all nine clusters.

Figure 1: ENVIRONMENT, STRESS, ALLOSTATIC LOAD, AND HEALTH OUTCOMES (A Complex Theoretical Map)

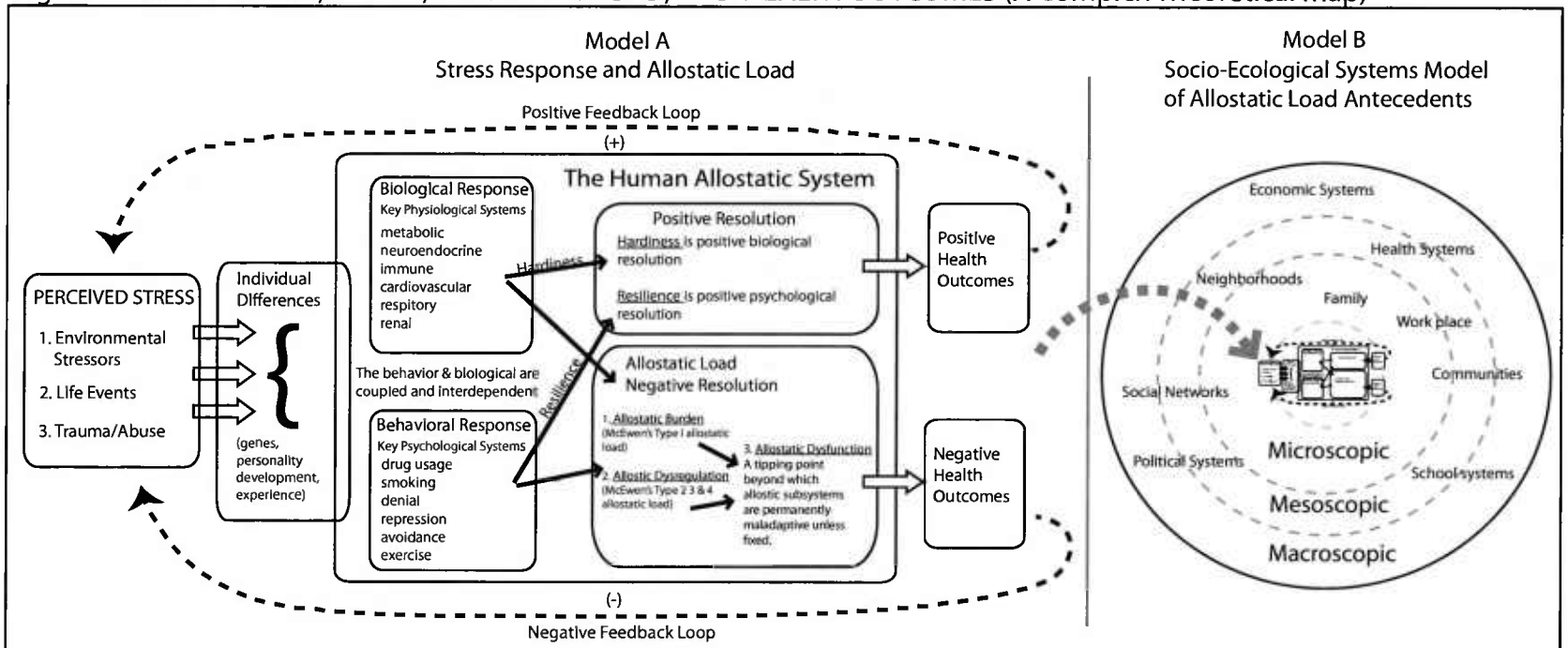
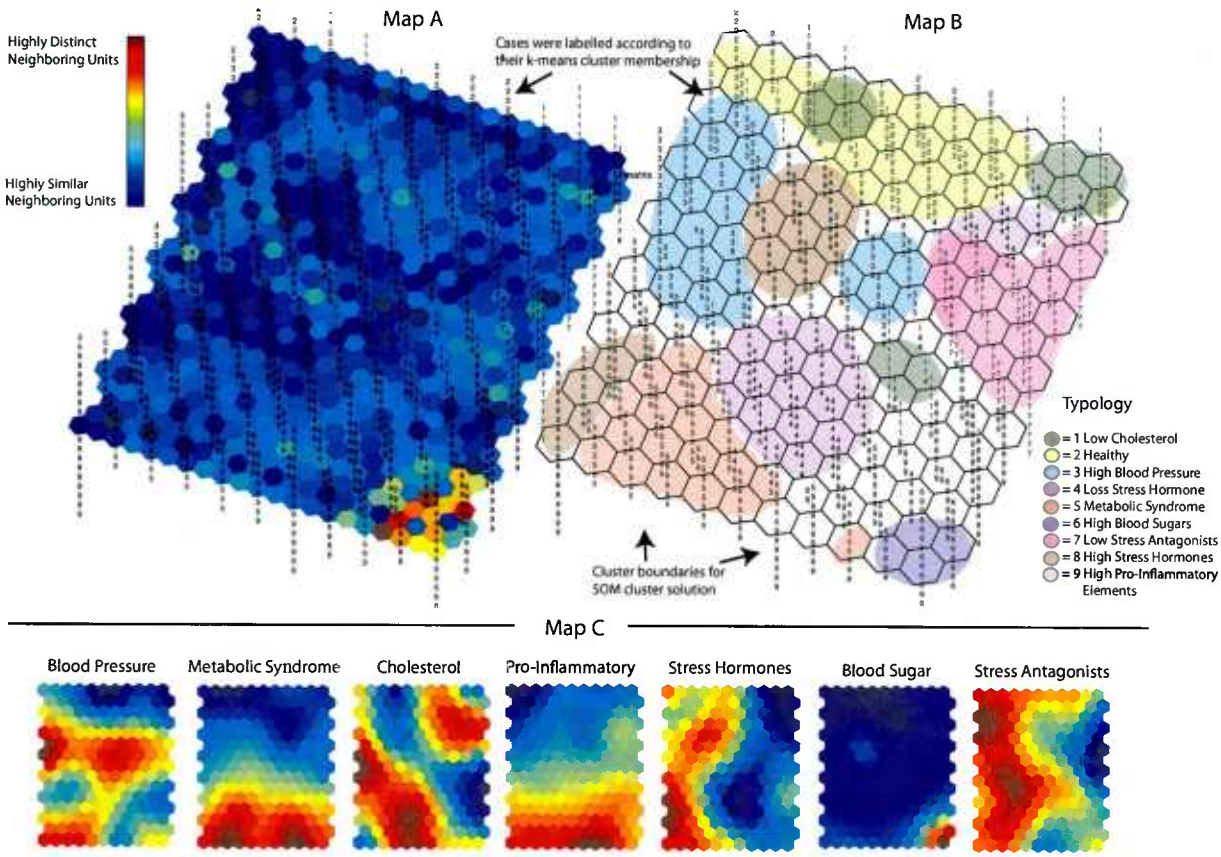


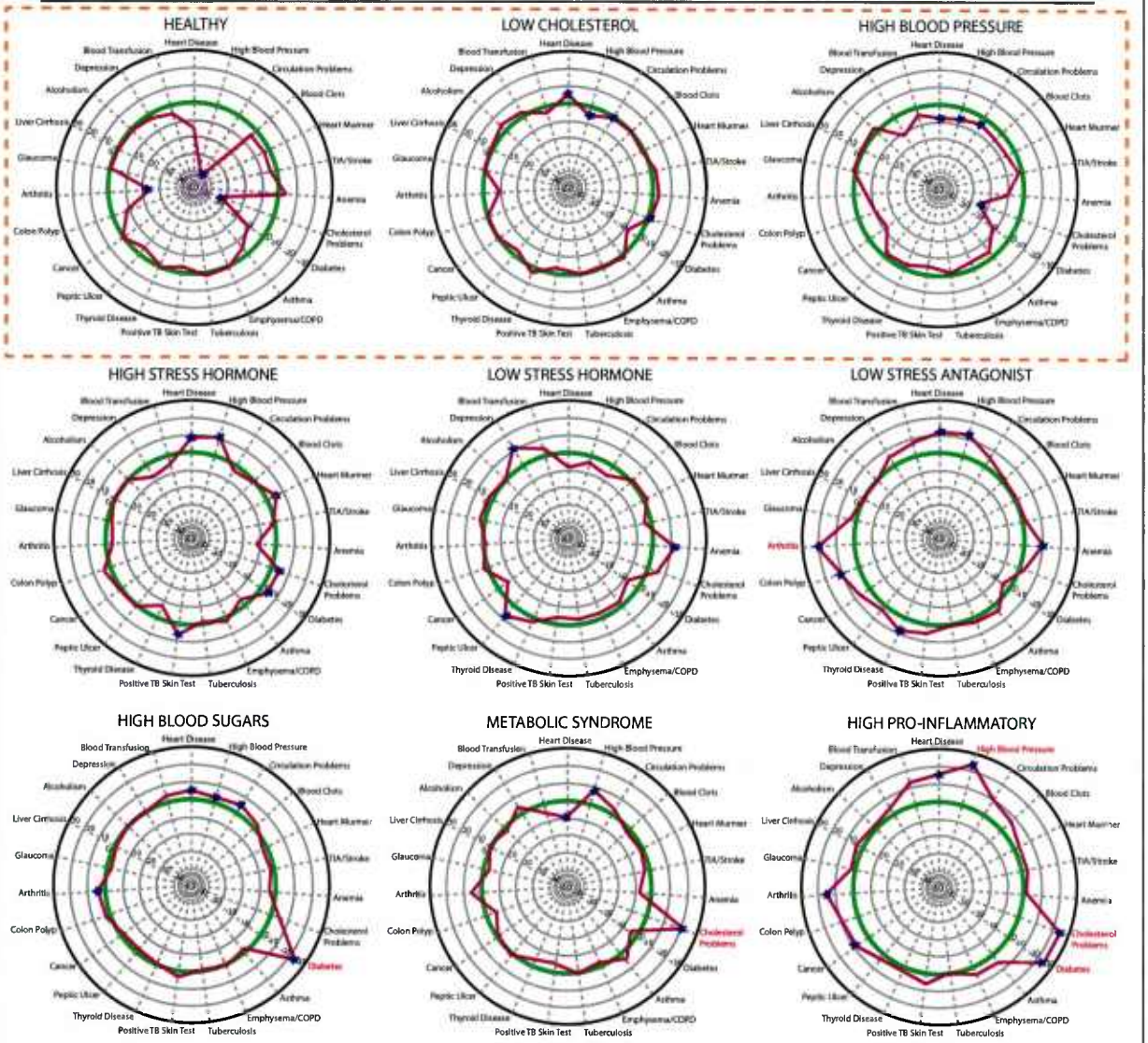
Figure 1 is a visual compilation of the theoretical frame outlined in ***Allostatic Load Biomarkers of Chronic Stress and Impact on Health and Cognition*** by Juster, McEwen and Lupien (2010), specifically, *Figure 2* and *Figure 6*. **Model A:** Based on Juster et al's Figure 2, it visualizes the stress response and allostatic load. See Juster et al for complete theoretical overview. **Model B:** Based on Juster et al's *Figure 6*, this model places *Model A* within a wider set of socio-ecological systems of key allostatic load antecedents. Juster et al got the idea from the work of Bronfenbrenner (See Refs). Moving outward from the microscopic (Model A) to the macroscopic, "the central propositions of Bronfenbrenner's general ecological model is that individuals are developmentally shaped by complex reciprocally interacting systems," which can have positive or negative effects on the stress and allostatic load of individuals" (Juster et al 2010, p. 15).

Figure 2: U-MATRIX AND COMPONENTS MATRIX MAPS FOR NINE ALLOSTATIC LOAD PROFILES



Map A and *Map B* are graphic representations of the cluster solution arrived at by the Self-Organizing Map (SOM) Neural Net, referred to as the *U-Matrix*. In terms of the information they provide, *Map A* is a three-dimensional (topographical) u-matrix: for it, the SOM adds hexagons to the original 15X11 map to allow for visual inspection of the degree of similarity amongst neighboring map units; the dark blue areas indicate neighborhoods of cases that are highly similar; in turn, bright yellow and red areas, as in the lower right corner of the map, indicate highly defined cluster boundaries. *Map B* is a two-dimensional version of *Map A* that allows for visual inspection of how the SOM clustered the individual cases. Cases on this version of the u-matrix (as well as *Map A*) were labelled according to their k-means cluster membership (The 9 cluster solution shown in Table 2) to see if the SOM would arrive at a similar solution. *Map C* is a graphic representation of the relative influence that the seven factors (shown in Table I) had on the SOM cluster solution. The SOM generates a mini-map for the seven factors, each of which can be overlaid across maps A and B. Each of these mini-maps can then be inspected visually to examine what its rates are across the different neighborhoods (clusters of cases). Dark blue areas indicate the lowest rates for a factor; and the bright red areas indicate the highest rates for a factor. For example, looking at the mini-map for Factor 6 (*Blood Sugar*), its rates are extremely low across most of the map, except for the lower right corner, which is where (looking at *Map A* and *Map B*) the SOM placed Cluster 6.

Figure 3: Clinical Outcomes (Health Risk Profiles) For Nine Allostatic Load Cluster Profiles



Radar Graphs of Case Clusters Across Outcomes. This Figure displays the differences between observed and expected frequencies for each self-reported medical condition. Each of the radii represents a self-reported medical condition, labeled at the top of their respective radius. The case clusters are circumscribed around the 23 points of each circle based on the average frequency on a particular self-reported medical condition. The resulting profile (which constitutes each Cluster's health risk profile) is in red. Score higher than 0 (the green circle) indicate a greater observed value than expected, whereas scores below 0 indicate a smaller than observed value than expected. For those scores higher than 20, the corresponding medical condition is labeled in red. The three healthy to marginally healthy profiles are at the top, outlined in orange.

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Change in Heart Rate Variability during Stress Resilience Training in a Virtual Environment

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Abstract

Power spectrum analysis of Heart Rate Variability (HRV) is a non-invasive procedure that allows for proxy study of emotional regulation by measuring autonomic nervous function. Virtual reality (VR) has become an increasing popular means of administering exposure treatments for anxiety disorders due to the added safety and ability to exert more control over the environment. The current data was extracted from a larger study developing resilience training for members of the armed forces using virtual combat scenarios in conjunction with a virtual mentor. To date, few studies have attempted to use HRV as an outcome measure in VR research. The ability to induce stress in a virtual environment has been well established, however, the corresponding change in HRV has not. The present study investigated whether the stress induced in VR creates expected changes in HRV. Additionally, the effectiveness of mentor training in increasing variability was investigated. Thirty-nine participants, including psychiatrically normal military cadets from navy/marine and army programs who had never been deployed as well as civilians, had fully usable data for analysis. The combat scenarios demonstrated significant difference from baseline using paired-samples *t*-tests. Overall, results support the use of HRV in VR interventions. Suggestions for improvement of mentor scenarios and directions for future research are included.

Change in Heart Rate Variability during Stress Resilience Training in a Virtual Environment

Emotions are embodied experiences, which result in varying degrees of physiological arousal. One of the most important systems in regulating this arousal, is the autonomic nervous system (ANS), which has two competing and complementary pathways: the sympathetic nervous system (SNS), responsible for increased arousal, and the parasympathetic nervous system (PNS), responsible for returning the organism to a healthy resting state following arousal. Adaptive emotional regulation requires an individual to change arousal levels relatively quickly as the demands of his or her environment changes. Conversely, emotional dysregulation results when the ANS becomes less flexible and thus unable to change the emotional and physiological state of the individual quickly (Appelhans & Luecken, 2006).

An obvious physical marker of increased arousal is increased heart rate (HR). Numerous factors, both physiological and environmental, influence an individual's heart rate. Heart rate variability (HRV) has been used as a marker of cardiac health for many years in the medical literature and in the past two decades or so, has gained increasing interest and use in the psychophysiological literature as well as a possible marker of autonomic health. HRV is a measure of how well an organism regulates cardiac rhythm in response to the changing demands of the environment. For the purpose of psychophysiology, the most relevant consideration is the influence of the ANS on heart rate. Both the SNS and PNS can affect heart rate and its variability, in a contradictory fashion. For example, a decrease in heart rate may result either from increased

parasympathetic activity or from inhibition of sympathetic activity (Appelhans & Luecken, 2006). Both the SNS and PNS influence heart rate by affecting the sinoatrial node, which is the area of the heart primarily responsible for pacemaking. Activation of the SNS has an excitatory effect on the sinoatrial node, resulting in increased heart rate. SNS activity is regulated via norepinephrine and works slowly. Conversely, the PNS, which affects heart rate via the vagus nerve, and is therefore often referred to as vagal activity, inhibits the sinoatrial node, resulting in decreased heart rate and works quickly (Appelhans & Luecken, 2006). The speed of vagal action is important in maintaining resting heart rate as the PNS has greater influence at rest, and keeps the heart rate below even the baseline firing rate of the sinoatrial node. When a person is healthy, primary control switches rapidly between the PNS and SNS, as they respond to the changing demands of the environment, resulting in a greater degree of variability. Yet when unhealthy, the person may become fixated in control by the SNS, without the proper inhibitory influence from the PNS, resulting in decreased variability (Appelhans & Luecken, 2006).

The most basic part of HRV measurement is the time in between beats, or from one beat to the next, generally referred to as the *normal-to-normal* (NN) intervals or RR intervals. According to the Task Force (1996), calculation of HRV is done by time-domain, frequency, or geometrically, though geometric calculations are complex and have not been commonly used in the literature.

The more commonly used method of analyzing HRV in the psychophysiological literature has been the use of power spectrum analysis of HRV, which requires only a nominal recording time of 5-minutes, and can be used with even shorter recording times.

In this method, ECG recordings are transformed, most often with a Fast Fourier Transformation. The resulting power spectrum shows a bimodal distribution with peaks in high frequency (HF) and low frequency (LF) ranges. The HF range occurs nominally at 0.15 – 0.40 Hz, which is the normal frequency of adult respiration, and has been well accepted as reflecting the influence of vagal activity on HRV. Furthermore, the HF range has often been considered synonymous with respiratory sinus arrhythmia (RSA), the natural variance in heart rate during inhalation and exhalation (Berntson et al., 1997). The LF range occurs nominally at 0.04 – 0.15 Hz. As opposed to the general acceptance of the physiological correlates of the HF range, controversy exists as to exact nature of the LF range. Due to this controversy, some researchers have argued for the use of the very low frequency (VLF; less than 0.04 Hz) as perhaps a better marker of sympathetic activity. However, this also remains in debate as little research has been conducted using VLF.

Several authors have argued for the use of HF and LF both separately as measures of PNS and SNS activity, respectfully, and use of the ratio of LF to HF as a measure of *sympathovagal balance* between the sympathetic and parasympathetic branches of the ANS on the heart (Malliani, Pagani, Lombardi, and Cerutti 1991; Pagani 1986). This view was echoed by the Task Force (1996), although the members of that group argued for a view of the LF as a product of both sympathetic and parasympathetic activity. Other researchers have argued that LF HRV does not reflect SNS activity but rather PNS activity, and cast doubt on the concept of sympathovagal balance (Eckberg, 1997; Reyes Del Paso, Langewitz, Mulder, van Roon, and Duschek, 2013). However, such critiques have failed to dampen enthusiasm for spectral power analysis of HRV as a non-invasive

way to measure ANS activity as it seems unequivocal that the frequency ranges measure different things. Reyes Del Paso et al. (2013) noted that although they believe all three frequencies are parasympathetically mediated, each is associated with other measures of cardiac activity differently. This finding, along with the admittedly dynamic nature of the ANS calls into question their argument that whatever they are measuring, it must be parasympathetic. Moreover, despite the wealth of evidence cited by critics, other researchers have found support for the concept of LF HRV as sympathetically mediated (e.g., Wheat and Larkin, 2010). Despite the controversy that exists regarding the exact nature of LF and HF HRV data, their continued use, as well as that of the LF/HF ratio remains justified. Regardless of whether or not LF can be seen as pure marker of sympathetic influence, it is clear from the literature that the power spectrum analysis of HRV is useful at least as a marker of autonomic flexibility. Even if the objections of some authors are accepted, and LF HRV should be conceptualized differently, that does not do away with the usefulness of power spectrum analysis of HRV.

Porges' (1995, 2001, 2009) theory of polyvagal control may provide the link between the apparent usefulness of LF/HF HRV separately and as a ratio, with the findings of critics that physiological blockage of the SNS does not result in the expected changes in LF HRV power. Porges noted that the vagus nerve is not a single nerve, but a collection of fiber bundles that actually originate in two distinct areas of the medulla. Moreover, he noted the vagus nerve is not solely efferent, but also contains many afferent systems that provide feedback from the viscera and heart and thereby affect emotion. Porges' argued that although the two vagal branches can act in concert, they need, and often do, not. The branches represent different types of vagal control on heart rate and

emotion regulation and sometimes may contradict each other. Porges' hypothesized that the bundle originating from the dorsal motor nucleus is phylogenetically older and responsible for automatic reflexes of the *viscera*. On the other hand, because of the many neuronal connections in the *nucleus ambiguus* the bundle originating there, which he termed the ventral vagus, is responsible for many things that are more particularly mammalian. Furthermore, this pathway, with its close proximity to the facial nerves allows for close interaction between the expression of primary emotions, such as fear, anger, disgust, and joy, and the vagal control of heart rate. Following Porges, it is possible that both HF and LF are in fact markers of vagal activity, as purported by critics. Yet, it may be that HF HRV reflects the increased inhibitory activity of the phylogenetically older dorsal vagus, while LF HRV reflects an increase in activity in the ventral vagus during novel stimuli. This makes sense of the fact that pharmacological blockage of the vagus nerve results in an overall decrease of the HRV power spectrum, while increasing the LF to HF ratio. Again, following Porges' theory of ventral vagal activity, it makes sense that LF HRV, if a marker of that activity, would often increase at times that SNS activity also increased (e.g., the introduction of novel stimuli), allowing LF HRV to still be a taken as a proxy of increased sympathetic activity.

Several studies have been conducted on HRV in PTSD. Cohen et al. (1998) found that those with PTSD appeared to be in a heightened state of autonomic function compared to controls such that their function at rest was equivalent to the function of controls under stress. Cohen et al. (2000) found that both individuals with PTSD and individuals with panic disorder had increased LF HRV and decreased HF HRV at rest as compared to controls. Similar to the findings from the prior study those with PTSD did

not show the normal decrease in HF HRV and increase in LF HRV during recall of traumatic events. Overall, these results indicated that persons with PTSD have a restricted range of ANS function and demonstrated that various anxiety disorders do not have identical effects on HRV.

Mellman, Knorr, Pigeon, Leiter, and Akay (2004) explored differences in the LF/HF ratio during rapid eye movement (REM) sleep in persons with a traumatic experience, within one month of the event. They found that those whom later developed PTSD had a higher LF/HF ratio during REM sleep than those who were not developing PTSD, suggesting increased sympathetic activation during sleep for those with PTSD, as soon as one month post event. Conversly, Nishith et al. (2003) found that female rape victims who successfully completed treatment and no longer met criteria for PTSD showed reduced LF/HF ratio during REM sleep following treatment.

Tan et al. (2009) examined HRV in a group of veterans who suffered from PTSD, mTBI, and/or pain in order to determine if the combination of multiple conditions would lower HRV beyond that of any one condition alone. They found that the SDNN was lower in this group compared with available norms, though only for all three conditions in combination. Tan, Dao, Farmer, Sutherland, and Gevirtz (2011) found that SDANN was significantly lower for the group of veterans with PTSD compared to control subjects, with a large effect size. The researchers further examined an HRV biofeedback paradigm, providing half of the PTSD group with eight HRV biofeedback sessions in addition to their ongoing treatment. When comparing the experimental group to the group that continued with only treatment as usual, the researchers found that the training increased the participants HRV and provided significant relief for combat related PTSD,

especially for the avoidance/numbing subscale of CAPS, whereas treatment as usual was connected with neither an increase in HRV nor symptom reduction.

Researchers have also examined HRV biofeedback in a group of community members in a substance abuse clinic who had elevated scores on a measure of PTSD symptoms (Zucker, Samuelson, Muench, Greenberg, & Gevirtz, 2009). Persons in both the respiratory sinus arrhythmia (RSA) biofeedback and the progressive muscle relaxation conditions had significant reduction in PTSD symptoms post-intervention, although the RSA group also had significant reduction in depressive symptoms and a trend towards reduction in substance craving, and demonstrated healthy levels of HRV

Thayer and his colleagues recently summarized their work on the inhibitory role of the prefrontal cortex (PFC) on autonomic function, particularly on HR and HRV (Thayer et al., 2009). In these studies, greater HRV was associated with better performance on continuous performance tasks, including a two-back task, under normal conditions (Hansen, Johnsen, & Thayer, 2003) and under threat of shock (Hansen, Johnsen, & Thayer, 2009), and the ability to inhibit a prepotent response (unpublished manuscript cited in Thayer et al., 2009). Furthermore, a manipulation of HRV via exercise regimen was found to increase executive function in a naval sample (Hansen, Johnsen, Sollers, Stenvik, & Thayer, 2004). In the threat of shock paradigm, although greater HRV was associated with better executive function, lowered HRV was associated with faster reaction time, perhaps as a result of hypervigilance. Other researchers have found that even subtle cues of threat may decrease HF HRV and this can impact cognition (Elliot, Payen, Brisswalter, Cury, & Thayer, 2011).

In a study on the effect of HRV during sadness, researchers found that HF HRV was predictive of cognitive reactivity and expression of dysfunctional attitudes when sadness was induced, and accounted for a unique portion of the variance beyond resting heart rate (Beevers, Ellis, & Reid, 2011). Denson, Grisham, and Moulds (2011) found that reappraisal in response to an anger-invoking stimulus increased HRV, whereas suppression or normal response did not increase HRV. Reynard, Gevirtz, Berlow, Brown, and Boutelle (2011) found that baseline HRV was predictive of persistence on an unsolvable anagram task among undergraduates and community members, so that those with higher baseline HRV were more persistent. Although a paucity, taken together, the results of research on emotions and HRV that exists have shown that increased HRV is a marker of greater emotional flexibility.

Lang, Davis, and Ohman (2000) proposed that negative emotions such as fear and anxiety are a result of motivational neural circuits which are responsible for survival. According to the authors, one important aspect of that circuit is the capability for startle. Furthermore, they argued that for persons high in negative affect, it is likely that they are hypervigilant and thus demonstrate an exaggerated startle response, especially in response to novel situations. They further proposed that continual anxious apprehension may be related to such heightened startle.

Ruiz-Padial and colleagues (2003) found that for those within the lowest quartile of HRV, there was a significant difference in startle response when exposed to pleasant images versus neutral or unpleasant stimuli, but no difference between neutral and unpleasant images. Results indicated that those with lowered HRV were unable to properly attune to non-threatening stimuli and, thus, were ever vigilant for perceived

threats. Other researchers have a found a moderating effect for HRV in women with bulimia, such that women with bulimic symptoms and low HRV showed an increased startle across all picture groups compared to women with bulimic symptoms and high HRV or controls, with the largest increase when pictures of food were presented (Rodríguez-Ruiz, Guerra, Moreno, Fernández, & Vila, 2012). Other researchers have found that fear-potentiated startle did not differ among those with anxiety sensitivityor panic disorder, whereas low resting HRV was predictive of increased startle. Moreover, persons with low resting HRV, regardless of diagnosis, failed to show a decrease in startle potentiation (Melzig, Weike, Hamm, & Thayer, 2009).

Besides the use of images, additional factors to enhance startle have been explored. Kamkwalala et al. (2012) tested the effect of HRV and darkness-enhanced startle in African-Americans who met criteria for PTSD. The authors noted that darkness is anxiolytic in most humans, but that the brain structure responsible for this effect is sexually dimorphic, as it is nearly 2.5 times larger in males than females. Results indicated that women with PTSD had a greater effect of darkness on startle magnitude than female controls. In addition, men with PTSD showed increased HF HRV throughout the session compared with male controls. The authors posited that these results indicated the possibility that the neural correlates of PTSD are sexually dimorphic, which may account for some of the various findings regarding LF HRV and HF HRV in the literature, especially when only one sex is studied.

Psychological research and intervention using virtual reality (VR) has grown exponentially in the past decade with most research supporting its usefulness (Buckwalter & Rizzo, 1997; Schultheis, Himelstein, & Rizzo, 2002; Schultheis & Rizzo, 2001).

Despite the financial and time costs associated with creation of the virtual environments several advantages exist for their use. It has been considered a safer, and often less threatening method of exposure therapy than traditional *in vivo* approaches. Numerous studies have used virtual reality exposure treatments (VRET) for phobias, such as arachnophobia (Côté & Bouchard, 2005; Garcia-Palacios, Hoffman, Carlin, Furness, & Botella, 2002), aviophobia (Maltby, Kirsch, Mayers & Allen, 2002; Rothbaum, Hodges, Smith, Lee, & Price, 2000) and social phobia (Anderson, Zimand, Hodges, & Rothbaum, 2005; Harris, Kemmerling, & North, 2002). Other interventions using VR have included re-training people after brain injury to be able to complete complex activities of daily living such as driving (Schultheis & Mourant, 2001) and grocery shopping (Rand, Weiss, & Katz, 2009; Whitney et al., 2006). VR also allows for a more standard presentation of research protocols than an in-person examiner can usually provide, especially when actors or confederates are required (e.g., Wallergärd, Jönsson, Österberg, Johansson, & Karlson, 2011). One possible issue however, is the need to maintain a sense of presence within the virtual environment. Relevant to technology- driven research and intervention, the International Society for Presence Research (ISPR) has defined presence (short for telepresence) as a "subjective perception in which even though part or all of an individual's current experience is generated by and/or filtered through human-made technology, part or all of the individual's perception fails to accurately acknowledge the role of the technology (ISPR, 2000)."

Wood and colleagues (Wood, Weiderhold, & Spira, J., 2010) used simple HR in a recent study of 30 service members diagnosed with PTSD and treated with VRET. They noted that HR corresponded well to patient rating on a Subjective Units of Distress

(SUDS) measure and they monitored HR in order to maintain an optimal amount of distressing activity within the virtual environment. The authors discussed the effectiveness of their intervention in two case studies, as one participant achieved a 24% reduction in PTSD symptoms as measured by the PTSD checklist-military version (PCL-M) and the other achieved a 63% reduction, with both being able to return to unrestricted active duty following treatment. Some strengths of their approach included using military language in order to assist with patient "buy-in." Namely, they referred to session as "hops" and "operational retraining" rather than therapy. Moreover, their virtual environment was constructed to allow for the therapist to increase certain factors or to place additional emphasis on factors that were more relevant to the specific case at hand.

In a review of VRETs, Meyerbröker and Emmelkamp (2010) noted that despite their popularity, only for a few disorders, namely fear of heights and fear of flying, have there been strong enough controlled trials to accept the effectiveness of VRET. VRET for other disorders such as panic disorder and social phobia have some research, but according to the authors, cannot yet be considered evidence based treatments. Moreover, they noted that for some disorders, such as agoraphobia, VRET is difficult to enact, due to patient unwillingness to leave the house, though they provided some suggestions for overcoming this difficulty. In all they noted that VRET appears to be a viable treatment option, but refinements can continue to be made and more rigorous methodology for controlled studies enacted.

Similar to goals of the current study, Côté and Bouchard (2005) sought to demonstrate the effectiveness of using psychophysiological data as an outcome measurement in VR research. Examining a sample of 28 adults with arachnophobia they

found that VRET had a significant effect on participant HRV measured in interbeat intervals (IBI) so that when baseline level was controlled for, there was a significant difference in IBI pre and post-treatment.

To date, only two VR studies were found that used frequency analysis of HRV as an outcome variable, and both were restricted to use of HF HRV. Wallergård and colleagues (2011) performed a study using a virtual version of the Trier Social Stress Test in which participants are required to deliver a speech and then solve an arithmetic task while in front of an audience, that, unbeknownst to them, are actually actors who have been told to not respond emotionally, making the test stressful for the participant. They noted that using a VR paradigm would assist in keeping conditions standard and avoided the cost of hiring actors. Amongst their outcome measures they included HR, HF HRV as a means of determining PNS activity, and transwave amplification (TWA), as a method of determining SNS activity, rather than LF HRV. The researchers found that the HR outcome was similar to *in vivo* TSST research outcomes. They also found significant results for changes in TWA, but failed to find any significant findings for HF HRV.

Pallavinci et al. (2013) used HF HRV as an outcome measure when seeking to determine if VR was effective when technological errors occurred. In other words, they sought to examine if technological problems noticed by the participants interrupted the feeling of presence and whether such interrupted VR stressors would differ from stressors caused by traditional media (i.e., audio, video, or text based). They confirmed the importance of presence and found that HF HRV differed significantly between groups so that interrupted VR was less effective at producing emotional stress than were traditional methods. One limitation of this study however was the failure to use a control condition

in which the same VR protocol was given without interruption. For the purpose of the present study two things were noted: one, the need to minimize technological miscues in order to avoid interrupting a sense of presence. More importantly, the HF component of HRV was demonstrated to be usable within virtual environments.

In the review cited above, Meyerbröker and Emmelkamp (2010) reported also on the use of psychophysiological components in some VRETs, including the study of Côté & Bouchard (2005) reviewed above. The authors noted that research on VRET using psychophysiological data has shown mixed results. Research to date that uses HR, HRV, and skin conductance research all have had noticeable flaws that limit the amount of usable information that can be taken from them and further research is needed.

Purpose of the Current Study

Given the underutilization of HRV as an outcome measure in VR research and the general lack of clarity regarding psychophysiology in VR noted by Meyerbröker and Emmelkamp (2010), the current study was undertaken in order to examine whether stressors caused by emotionally laden material presented within a virtual environment cause a significant change in HRV. A white-noise startle paradigm, without virtual environment, was used as a control, and was expected to cause similar increase in HRV from baseline as shown in past studies, with attenuation of effect expected in the second administration (Hypothesis 1).

The research on VRET for anxiety disorders, including PTSD, has demonstrated the usefulness of virtual reality stress induction. However, such studies have only demonstrated that virtual reality can induce stress via exposure for groups for whom the virtual environment and its stressors are particularly salient. The present study extends

prior research by determining if HRV changes are possible within virtual environments for groups for whom the virtual environment is not related to a pre-existing psychiatric diagnosis (e.g., PTSD or specific phobia) and by utilization of both LF and HF HRV and their ratio, as opposed to IBI or HF HRV alone. Assuming participants are sufficiently present within the virtual environment, a similar change in HRV measurement was expected as those demonstrated in other studies with emotionally laden stressors shown in pictures or videos (Hypotheses 2-6).

Although little empirical evidence exists to support specific hypotheses regarding the relative differences in HRV caused by differing emotional stimuli present in the scenarios, taking a more qualitative approach, some hypotheses can be made that fear, guilt, and startle will cause more change from baseline, whereas passive watching of events will cause less change from baseline (Hypothesis 7). A final intent was to examine if the mentor training had any demonstrable effect on participant stress, such that participants receiving mentor training would demonstrate decreased sympathetic activation as evidenced by lower LF/HF ratio levels in scenarios 3 and 4, once the participant had received adequate training (Hypothesis 8).

Hypotheses

Hypotheses for the current study are as follows:

1. For all participants, LF HRV will increase and HF HRV will decrease compared to baseline when auditory startle is induced, leading to an increase of the LF/HF ratio. This will be most notable during the first administration of the startle-inducing stimuli, and will be attenuated for the second administration of the stimuli.

2. For all participants, LF HRV will increase and HF HRV will decrease, leading to an increase of the LF/HF ratio as well, compared to baseline in Combat Scenario 1 following the discovery of the body as the participant waits tensely and is forced to consider the morality of allowing the man to die.
3. For all participants, LF HRV will increase and HF HRV will decrease, leading to an increase of the LF/HF ratio as well, compared to baseline in Combat Scenario 1 following the startle induced by the explosion.
4. For all participants, LF HRV will increase and HF HRV will decrease, leading to an increase of the LF/HF ratio as well, compared to baseline in Combat Scenario 2 following the startle and fear induced by the explosion.
5. For all participants, LF HRV will increase and HF HRV will decrease, leading to an increase of the LF/HF ratio as well, compared to baseline in Combat Scenario 3 following the anger and frustration induced by witnessing the beating of the woman.
6. For all participants, LF HRV will increase and HF HRV will decrease, leading to an increase of the LF/HF ratio as well, compared to baseline in Combat Scenario 4 in the two minutes following the startle, fear, guilt, and sadness induced by the explosion and death of the boy.
7. Regarding specific emotions, it is hypothesized that fear for one's own safety along with startle will likely produce the largest change from baseline (explosion in scenario 2), startle with sadness and guilt will produce the second largest change (explosion in scenario 4), and startle without fear will produce the third largest change (explosion in scenario 1). Regarding the

discovery of the body in scenario 1 and the witnessing of the beating in scenario 3, it is hypothesized that although these will still be significantly changed from baseline, they will be relatively less changed from baseline due to the passive nature of the interaction.

8. There will be a significant group effect such that those receiving mentor training will show decreased sympathetic activation as measured by the LF/HF ratio than those who do not receive such training.

Method

The current study was part of a larger, ongoing study by a large Southern California research university to investigate ways to train resiliency for United States military service members scheduled to be deployed in the future into hostile fire combat zones. The current study was drawn from data from the first of three research experiments planned, which involved recruitment of a small sample as a pilot study in order to assess the functionality of the equipment and the usability of the training modules produced.

Participants

Forty-seven participants completed the procedures. Study participants were recruited via fliers and emails from the Recruit Officer Training Corps (ROTC) of a large Southern Californian university; civilian students, staff, and faculty from that same university, and cadets from the United States Military Academy. Participants were excluded if they did not have normal or correctable vision, as they would not be able to use some of the required equipment. As the intended population for study was military members who were likely to see direct combat action, persons younger than 18 years of

age or over age 45 were also excluded. Furthermore, in order to ensure no participants had pre-existing combat stress, participants must have had no prior deployments or combat history. Given the hierarchical nature of the military, additional safeguards were installed to ensure that potential participants serving in the military did not feel coerced into participating. Specifically, eligible participants were provided information and given the chance to provide consent or refusal to participate without noncommissioned or commissioned officers in their command present.

Procedures

Participants were brought in individually to engage in the Stress Resilience in Virtual Environments (STRIVE) protocol. They engaged in four developed virtual combat scenarios (CS) and were assigned to one of two groups. Scenarios for the experimental group included the virtual mentor, who provided the participants with psychoeducation about the physiological stress response and trained the participants in use of Cognitive Behavioral Therapy (CBT) stress resilience techniques. Scenarios for the control group were identical, but with the mentor component removed. Although true random assignment was not possible given the collection procedures used, quasi-random assignment of placing every participant in the opposite group as the last participant. This was used in order to keep groups numerically even.

Participants initially filled out several questionnaires prior to engaging in the virtual environments. The participants were then fitted with the BIOPAC MP150, a chest-mounted device, which measured electrocardiogram (ECG), respiration, and galvanic skin response (GSR). Some participants were equipped with other psychophysiological equipment, not pertinent to the current study. Following that, participants were equipped

with a Sony HMZ-T1H HMD fitted with an Intersense InertiaCube^2 and a neoprene HMD "shield" to block out other light. For the first two combat scenarios, participants were seated and used a Logitech Rumble Game Pad F510 to drive their virtual Humvee. During the final two scenarios, participants stood and carried a mock training M16A1 rifle to increase immersion in the environment as forward and backward movement were predetermined in those scenarios. Scenarios were run on an Intel Core i7-2600 8-core CPU running at 3.4 GHz with 8 GB of RAM using two nVidia GeForce GTX570 graphics cards in an SLI configuration.

Upon being equipped with the psychophysiological and virtual reality equipment, there was a 2-minute period during which the screen was dark and silent, prior to engagement in the virtual scenarios. The first minute was completely silent in order to determine the participant's resting heart rate, HRV, respiration, and GSR. At approximately a few seconds past the 1-minute mark, the participant was subjected to a blast of "white noise" in order to ascertain the change in their physiological responses from a "pure" startle. The final minute was similar to the first and was included as a recovery time to return to baseline prior to the beginning of the Combat Scenario 1. The participant then engaged the first two combat scenarios as well as mentor sessions for those so assigned (combat scenarios and mentor sessions are described below). Following the first two combat scenarios, the game pad was removed and the participant was given a mock rifle for the final two scenarios. Prior to the start of Combat Scenario 3, another 2-minute period of darkness identical to that preceding Combat Scenario 1, including noise-induced startle, was presented. After the end of Combat Scenario 4, a final 2-

minute recovery period without startle was presented, but no psychophysiological data was recorded.

Following engagement with the virtual environments, participants filled out a post-questionnaire and were provided $40 compensation for their time. Total time for participation was approximately 2.5 hours including 15 to 25 minutes to answer initial questionnaires, 40 to 60 minutes to be equipped with psychophysiological and HMD equipment, 30 to 60 minutes to engage in the virtual environments (depending on group), and 10 to 20 minutes for the post assessment.

Measures

As part of the larger study, participants filled out several questionnaires including a demographic questionnaire and a post-questionnaire, intended to allow participants to provide feedback about the usability of the system. The demographic questionnaire included items about age, ethnicity, education and gender. Other questionnaires were not pertinent to the present study.

Virtual reality modules. Participants underwent four virtual modules that included Combat Scenarios 1 through 4. Half of the participants were also exposed to the mentor sessions that corresponded to each combat scenario. Lengths of scenarios and mentor sessions varied, with each module lasting approximately 15 minutes for the mentor group. Within mentor sessions 2 and 4, a SUDS was used to measure participant reaction to the scenario. The scale was a number from 1 to 10, with 1 being absolutely no distress and 10 being the most distress imaginable. Content of the virtual scenarios was slightly different for Marine and Army personnel, as the virtual characters were dressed differently (in Marine desert digital camouflage or the Army combat uniforms) and

minimally different dialog was used to correspond to the different branches of service. Civilian participants received the Marine version of the scenarios.

Combat Scenario 1. The participant was given an introduction, which informed the participant of his or her role as the driver for the team and was instructed to drive a Humvee down a stretch of road in Afghanistan with the rest of the team, while maintaining a given interval between themselves and the vehicle in front of them as a task to keep them engaged in the virtual scenario. Their mission was to drive to town and gather information. However, before the participant reached the town, a body was seen in the middle of the roadway, and the convoy stopped. Upon realizing that the man is still alive, the participant listened to a discussion between the other team members about whether to help him or not, due to the possibility of a booby trap. The man dies prior to Explosive Ordnance Disposal (EOD) coming to determine the presence of a trap. For those so assigned, the mentor session began once EOD arrived and sent a robot to investigate the body. For those not assigned to the mentor group, the scenario continued with the revelation that the body was indeed booby trapped as a large explosion engulfed the EOD robot, and the participant continued directly into Combat Scenario 2.

Mentor Session 1. Captain Branch, the virtual mentor in all scenarios, introduced himself to the participant within the Humvee he or she had been driving and explained how preparing for the mental aspects of combat was as important as the physical preparation and training the service member had already accomplished. The mentor then explained that difficult moral decisions such as the one shown in Combat Scenario 1 were an unfortunate reality in war. The combat scenario was continued, so that participants in the mentor group also learned that the body had, in this case, been booby trapped.

Following another brief interaction with the mentor, the session ended and combat scenario 2 began.

Combat Scenario 2. As the convoy continued into the village, concern about possible lookouts was discussed by the vehicle commander. Just as the convoy exited the small village, a roadside explosion occurred, which damaged the Humvee that the participant was driving, rendered the gunner unconscious and caused the rest of the characters in the scenario to react in various states of anxiety, including panic. For those so assigned, Mentor Session 2 began. For the control group, equipment was changed and Combat Scenario 3 began.

Mentor Session 2. The scene changed to a military classroom, where the mentor showed the participant how the human body reacts to stress through an anatomically accurate visual animation of the body's stress response including neurologic, adrenal, pulmonary, and cardiac changes. The mentor showed a video clip of Combat Scenario 2 (a replay of the roadside explosion and its result) and then administered the SUDS with regard to how the participant felt during that event in the scenario. The results of the SUDS was then compared to a comparable scale that the computer automatically calculated based on the participant's actual psychophysiological response (i.e., heart rate, respiration, and skin conductance). The participant was then taught a deep breathing exercise consisting of inhaling through the nose for four seconds, holding for one second, and breathing slowly for five seconds, which resulted in breathing rate of approximately six breaths per minute. After practicing the breathing exercise, participants engaged in an interactive practice session that used the participant's real-time respiration rate to give visual and auditory feedback with regard to their performance on the breathing exercise.

Combat Scenario 3. Following the change of equipment and 2-minute black period, the participant was given another introduction, which informed the participant of the new role as part of a walking patrol in a city with the same team. The participant was instructed to stay behind the team leader and visually scan rooftops, windows, and doorways for possible threats (although no threats actually emerged) in order to keep them engaged in the virtual scenario. After walking a short distance, the team came upon a woman being beaten by several males. The team became angered, and the team leader instructed their interpreter to make the men stop. After several tense moments, the participant learned that the woman was accused of adultery and would be exiled following the beating. Although the team leader was displeased, he instructed the interpreter to tell the men that the beating had gone on for long enough and to let the woman go into exile if that was their custom. At this point, the mentor session began or the walking patrol continued in Combat Scenario 4.

Mentor Session 3. Similar to Mentor Session 1, the mentor appeared on the scene to discuss the moral implications of what they had allowed to occur and that, though the American way of life was good for Americans, it was not the place of United States service members to change the culture of the country they served in, regardless of personal distaste for some actions in that culture, and that the mission comes first.

Combat Scenario 4. The participant entered an Afghanistan marketplace with the team, in order to talk with a civilian elder. The elder became quite irate, telling the team they needed to leave immediately. Subsequently, the crowd around began yelling at the team. As the discussion with the elder continued, a civilian boy kicked his soccer ball to one of the participant's team members, who kicked it back off-target, and it accidentally

went into an alley. As the civilian boy chased the ball into the alley, he tripped a pressure-plated Improvised Explosive Device (IED) that was meant for the team and that the elder had been trying to warn them about. The boy was severely injured, and despite the efforts of the participant's squad members, died. The participant witnessed the team leader trying to convince the team member who kicked that ball that it was not his fault, and finally watched as the mother cried over the boy's body. For those assigned to the mentor group, the session began. For all others, the 2-minute silent recovery period began.

Mentor Session 4. The mentor again appeared on the scene and, through an anatomically accurate visual animation, showed the participant how the pre-frontal cortex and amygdala interact. The mentor then showed a video clip of the explosion from Combat Scenario 4, administered the SUDS, and compared the participant response to the actual psychophysiological data, similar to the procedure used in Mentor Session 2. The participant was then given a lesson on mental awareness using a five level scale of readiness. The participant was encouraged to considering possible scenarios they may encounter in combat and engaged in an exercise to consider the proper level of readiness in various situations. The participant then had the 2-minute silent recovery period and the experiment ended.

Psychophysiological data. Data for HRV was obtained according to the guidelines set forth by the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (1996) using frequency analyses, as this has been considered the best way to determine HRV from relatively short (approximately 5 minute) recordings of ECG data. HRV data was obtained using

the BIOPAC MP 150 which had channels for GSR (GSR100C), ECG (ECG100C), and respiration (RSP100C).

Results

Preliminary analyses

Of the 47 participants who completed the procedures, six had to be excluded due to failure of equipment to record psychophysiological data. Two additional participants had partial data recorded, but were also all removed from all analyses to allow for consistency in the sample and to ensure that possibly corrupted data was not used in the analyses. Unfortunately, these failures were not evenly distributed across the two conditions. Thus, data from a total of 39 participants were analyzed: 22 in the mentor condition, and 17 in the non-mentor condition. Military affiliation was roughly even with 21 cadets and 18 civilians participants.

All recording times, with the exception of the baseline and startle times were 2 minutes. Baseline and pure startle recording times were 1 minute each. Data were extracted using an in-house custom designed MATLAB program. HRV data was visually inspected for artifacts and ectopic beats using Acqknowledge software. All such artifacts were either deleted or replaced using cubic splicing, depending on suitability of surrounding data (Task Force, 1996). The recordings then underwent fast- Fourier transformation for power spectrum analysis. Transformed data was visually inspected for outliers. One outlier was identified and changed to the average of the other scores in that recording period. All power spectrum analyses were performed with data expressed in normalized units, per Task Force (1996) recommendations.

Data was analyzed using PASW version 18. Independent samples *t*-tests were used to determine if groups differed on HRV or if military members differed from civilian participants on HRV. The findings revealed no significant differences between groups on these variables. Hypotheses 1 through 6 were tested using paired sample *t*-tests with each participant acting as their own baseline. Hypothesis 7 was tested using paired sample *t*-tests between scenarios and visual examination of mean difference. Hypotheses 8 was tested using mixed-method ANOVAs.

Most HRV ratio data was somewhat skewed, generally in the positive direction, with most scores clustered near the negative end of the distribution. Logarithmic transformation was considered, but rejected as ANOVA is relatively robust to violations of normal distribution with $N > 30$. After running initial analyses as planned, logarithmic transformation was used and analyses re-ran in order to ensure that the violation of normalcy had not had a significant effect on the results. Results of that analysis demonstrated no significant differences from original analyses.

Analyses

Change in HRV from baseline (Hypotheses 1-6). Study hypotheses were partially supported. LF/HF ratios for white noise startle ($p = .006$), CS1 body discovery ($p = .01$), CS2 explosion ($p = .04$), CS3 beating event ($p > .001$), and CS4 explosion ($p > .001$) all demonstrated significant change from baseline in the expected direction. Only the ratio for CS1 explosion event failed to demonstrate a significant difference ($p = .18$). However, contrary to study hypotheses, there was not a significant attenuation of effect for pure white noise startle $t(38) = -1.16$, $p = .25$. Table 1 reports means, standard deviations, and *t* values for all test variables from Hypotheses 1 - 6.

Given the change in body position from CS1 and CS2 to CS3 and CS4, an additional analysis was run to compare results for the second startle and CS3 and CS4 with the second baseline. This was in order to ensure that found results were not confounded by the change from being in a seated position to a standing position which, as mentioned above, occurred after CS2. Mean baseline ratios when seated or standing were not only not significantly different ($p = .47$), but were nearly identical (CS1 baseline $M =$ 0.77, CS3 baseline $M = 0.79$). Moreover, all variables were found to remain significant at a low level of chance ($p > .001$ for all variables) indicating that the change in position was not likely responsible for the noted effect. Table 2 reports means, standard deviations, and t values for these analyses.

Changes relative to specific emotions (Hypothesis 7). Regarding specific emotions, changes partially supported study hypotheses. Comparing scenarios to each other, rather than simply to baseline, significant differences were found between CS1 body discovery and CS3 beating event ($p = .009$), CS1 body discovery and CS4 explosion event ($p = .02$), CS1 explosion event and CS3 beating event ($p = .006$), CS2 explosion event and CS3 beating event ($p = .048$), and CS2 explosion event and CS4 explosion event ($p = .006$). Looking at mean differences from baseline, it was noted that the CS4 explosion event had the largest change, followed by the CS3 explosion event. Next, CS1 body discovery and CS2 explosion had nearly identical differences, and CS1 explosion had the smallest difference. Table 3 provides means, mean differences, and t-values for these analyses. Figure 1 shows the relative change from baseline for all participants in all scenarios.

Mentor group (Hypothesis 8). Study hypotheses were not supported for the mentor group. For combat scenario 3, there was neither a significant main effect ($F(1,37) = 0.807$, $p = 0.38$) nor interaction effect ($F(1,37) = 0.164$, $p = 0.69$). Similar results were obtained for combat scenario 4, with neither a significant main effect ($F(1,37) = 0.084$, $p = 0.77$) nor interaction ($F(1,37) = 0.823$, $p = 0.37$). Figure 2 shows the relative change from baseline for participants split by group for all scenarios.

Additional Analyses. Some researchers have found that a baseline LF/HF ratio of greater than 1.0 indicates a restricted range of variability (thus a higher degree of emotional dysregulation) in participants such that they are less able to demonstrate changes in variability in study protocols (Hart, personal communication). Seven such participants were found in the present study. In order to determine the effect that these participants may have had on results, analyses were re-run with those participants excluded from the additional analyses. Thus, 32 participants were in these analyses with 19 in the mentor condition. Results were largely similar with some noted changes. First, the CS1 explosion event demonstrated a significant change from baseline in the expected direction ($t = -2.922$, $p = .006$). Second, not only did pure noise startle response still not attenuate, it actually demonstrated a significant increase from time 1 to time 2 ($p = .007$), which was not expected. Finally, when it came to relative differences between scenarios, only the differences between CS1 body discovery and CS3 beating event ($p = .009$), CS1 explosion event and CS3 beating event ($p = .006$), and CS2 explosion event and CS4 explosion event ($p = .006$) remained significant. Mean differences remained in the same order, but with the CS2 explosion event demonstrating less change from baseline than CS1 body discovery, rather than being similar. Significance for all other results were

unchanged. Tables 4, 5, and 6 provide results of these analyses comparable to Tables 1-3. Figures 3 and 4 provide similar results as Figures 1 and 2, for this truncated sample.

Discussion

Virtual Reality, Emotions, and HRV

Few research studies to date have used HRV in virtual environments and those few have had mixed results. Wood and colleagues (2010) found that simple HR was useful as a measure of objective distress and could be used in a virtual exposure training to tailor individual sessions for maximum effectiveness. For HF HRV, Wallergärd et al. (2011) found that a social stressor had did not significantly effect it, though they failed to discuss the implications of this finding in their article. Pallavinci et al. (2013) did find differences in both HF HRV and in transwave amplitude (a measure of sympathetic response), but noted that within the virtual environment, these changes were less than during other forms of media when the VR program had technical problems which were noticeable to the participant. However, they chose not to have a control condition wherein participants received the VR training without technical problems and this limits the usability of their findings. In the most similar study to the current one, Côté & Bouchard (2005) demonstrated that a virtual stressor could affect interbeat intervals.

The current study lends more support to the latter finding of the usability of measures of HRV in virtual environments and specifically adds to the literature a notable case of the usability of power spectrum analyses in virtual environments. Virtual reality induced emotional states effected HRV generally as expected, and this supports the use of HRV power spectrum analysis as a valid method to determine to effectiveness of emotional stimuli presented in a virtual environment.

The current study also provided several additional lessons regarding HRV in virtual environments. As noted, when all participants were included regardless of baseline LF/HF ratio, the explosion event in combat scenario 1 was not significantly different from baseline. Although, significance was demonstrated with healthier sample, there is still the question of why this event was the only one to be non-significant in the original analyses. It was expected that explosions within the scenarios would be somewhat comparable to the pure white noise startle induced following baseline, with other emotions induced by the scenario having a possible additive effect. It may have been that with regards to combat scenario 1, the explosion was not as surprising. In real life, a robot is by EOD sent to investigate suspicious items/events precisely because those things may explode. It seems likely that the events leading up to the explosion in combat scenario 1 prepared the participants somewhat for the explosion, and thus, made a startle response less likely. However, it should be noted that with those with restricted ranges of variability removed, even this less surprising explosion created a significant startle. Future research using HRV in VR should make certain to include considerations of participant baseline HRV in planning and consider a baseline LF/HF ratio higher than 1.0 as an exclusionary criterion for research designs without an intervention component.

Regarding attenuation of pure startle, other researchers have shown that startle response does not always attenuate (Melzig et al., 2009). However, in the current study, there was not only a lack of attenuation, but with restricted range participants removed, there was actually a significant increase in startle response. This was not expected, but could possibly be explained by several factors. One was the participant change from sitting to standing. Although resting baseline LF/HF ratio while sitting or standing was

nearly identical in the full sample, it makes sense that standing up could theoretically make one more ready to act on a given threat and thus to demonstrate an increased startle response. Another could have been that, due to the emotionally laden material in which they received other startles (i.e. explosions) in the first two scenarios, participant startle response was heightened. Although it is highly unlikely that virtual environments could cause clinical PTSD, given the military nature of the scenarios and the fact that exaggerated startle response is a common symptom of combat exposure, it may have been more prudent to expect that an exaggerated startle response could be induced as was shown.

There was little empirical evidence to determine specific hypotheses for relative differences among the various scenarios and their associated emotional states. Therefore, that results were not exactly as hypothesized is unsurprising, but some further reflections can be made. Significant differences found between scenarios regardless of participant pool used (i.e., all participants or low-baseline ratio only) were always in the direction that events in CS3 and CS4 showed increased change compared to CS1 or CS2 events. Thus, the possibility that switching to an upright body position was a confounding variable for this hypothesis cannot be ignored, especially given that medical research with HRV often contains a body position change specifically to create a change in HRV (Task Force, 1996).

Even so, it is important to consider the implications of the results if accurate. The CS4 explosion event, CS3 beating event, and the CS1 body discovery event demonstrated the largest change from baseline. Given that the passivity of CS3 and the CS1 body discovery were expected to produce the lowest change, this is somewhat surprising. It

may be that the anger and frustration of feeling unable to act to change the outcome in any of these events may have led to a relatively larger increase in the LF\HF ratio. Some support for these results can be found in the results of Denson and colleagues (2011) as well as Reynard et al. (2011). Denson and his co-researchers the results showed that reappraisal following an anger-inducing scenario increased the ratio, which is similar to what the participant is required to do in CS3. Regarding Reynard et al., the finding that those within the lowest quartile of baseline HRV persisted longer on an unsolvable anagram, while not precisely similar to current results, does demonstrate a significant link between HRV and frustration tolerance, which may help explain the results found here. In any case, the results in this area for the present study, though not entirely clear, do lend support for continued investigation of HRV in specific emotional states, especially regarding the effect of increased frustration. Moreover, given that frustration and a sense of loss of control can be a common experience of service members on deployment, the effect of these sources of frustration on participant HRV in the larger ongoing study from which this study was drawn will be important.

Mentor intervention

Counter to study hypothesis, the administration of the mentor scenarios produced no significant results when compared to those who received no such training. This may indicate that although the present results demonstrated the usability of HRV as a manipulation check for emotion in VR studies, its use as an outcome measure may require more refinement. Additionally, several other factors may help explain the lack of significant results, and these may allow for improvement of the protocol in the future planned studies.

The mentor is intended as a pre-deployment intervention to increase resiliency via provision of knowledge about potential traumatic events and increased skill in coping with such scenarios. As such, the combat scenarios were created with intent of being integrated with the mentor sessions. Thus, in retrospect, it may have been to attempt to examine whether the mentor had an effect on participant HRV by comparing a mentor group to a group that received the combat scenarios, but no training. Although running participants in this way was initially conceived as a possible way to create a control group for future studies, it inadvertently had several disadvantages. For example, there was no true post-intervention outcome. Although a return to baseline after CS4 was administered, issues in programming did not allow for accurate measurement of HRV during this time. As such, the mentor group was compared to the non-mentor group while still undergoing the intervention, rather than after completion. Ensuring adequate measurement of the post-intervention return-to-baseline period might allow for better comparison between the groups.

Additionally, the time taken by the mentor could not be replaced with a placebo VR intervention due to the significant time and financial cost of developing virtual scenarios. One difficulty that resulted was that the non-mentor group finished the scenarios in approximately half the time of the mentor group. Conceivably, this may have had an effect on short-term changes in HRV due to the sometimes unpleasant nature of the VR equipment (e.g., heavy HMD). Wallergärd and colleagues (2011) noted that HMD use can be associated with reduced presence in virtual environments compared to large scale virtual environments which do not require such equipment. Thus any treatment effects gained may have been unrecognizable due to the increased time and

frustration possibly experienced by the mentor group. In light of these considerations, it may simply be wisest for researchers to use a different form of control group in future studies, especially studies are intended to examine pre/post deployment groups. One possible suggestion would be the use of a treatment as normal group (i.e., a unit that undergoes normal preparation for deployment) versus the intervention group. Choosing units being deployed to similar locations would also help reduce confounds.

Another consideration is the fact that the four combat scenarios used in the present study are not the only scenarios being produced and it is possible that the mentor effect will become more noticeable with increased sessions. Conversely, as increased sessions will require more time in the virtual environment, the increased LF/HF power that could occur from this artifactual frustration may continue to mask intervention effects. Although it may difficult to accomplish practically, changing to a method of intervention administration that requires no more than 30 minutes at a time in the virtual environment may relieve these concerns. Moreover, such an approach could have the added benefit of allowing service members time to master the one or two skills/knowledge points addressed in each scenario before moving onto others rather than having all such information presented at once.

Perhaps most importantly, it must be recognized that the scenarios were not intended primarily as a means to change HRV. Only the six-breaths-per-minute training which occurs in the second mentor session is particularly similar to the HRV biofeedback paradigms used by researchers. Lehner and colleagues (2011) also stressed that although this rate of breathing is close to the resonant rate for most people, it is insufficient to train people like this if the goal is to increase their HRV. In the larger study from which this

data was drawn, although HRV was a variable of interest, the overall outcome goal was to increase stress resiliency, of which, improving ANS healthy functioning via increased HRV is only one possible goal. Still, future studies in this series could seek to more fully incorporate biofeedback paradigms in later sessions and/or in combination with non-VR protocols if change in HRV function as a means to increase physiological and psychological resiliency is a priority.

The findings of Wood et a. (2010) regarding their VRET for PTSD demonstrated the importance of having flexibility within a virtual environment to make it as meaningful to the individual as possible. In keeping with those findings, one strength of the current protocol was the use of relevant language and equipment in the virtual environment according to the participant's affiliation with the military, including the mentor's uniform. Although this might be thought to be unimportant, use of another branch's jargon and uniform would likely significantly impact immersion in the environment by members of the other armed forces and this would almost certainly harm participant sense of presence and acceptance of the training.

Limitations and Directions for Future Research

The present study should be discussed in light of several limitations which may have impacted results. First, this pilot study involved a relatively small number of participants, several of which were lost to missing or incomplete data and which resulted in uneven distribution of participants between groups. The small sample size may have a created a lack of statistical power to reveal significant effects of the mentor intervention. Furthermore, the lack of true random assignment, though common in psychological research, may have had an unknown effect on outcomes. Future studies in this series of

experiments should seek to approximate random assignment more closely, if possible. Additionally, due to some initial difficulties with equipment, some protocols were changed slightly during the course of the experiment in order to ensure better performance. Although this was not expected to have created a noticeable change in outcome, it does represent a possible confound. However, this is less likely to remain a problem in future studies as these issues were largely mitigated during the present pilot study. Finally, although somewhat common in psychophysiological research, and necessary for the present study aims, the Task Force (1996) noted that short recording times for HRV can be problematic, especially given the sensitivity of HRV measurement to movement and other artifacts. However, the present study used chest-mounted equipment to measure HRV, which is intended to be less sensitive to movement and other artifact than are other forms of measurement such as those placed on the extremities. Moreover baselines were taken whenever participant changed to a significantly different body posture and the lack of significant differences between baselines helped alleviate concerns about movement artifact, and demonstrated that HRV can be used even in protocols that require participant movement if sufficient care is taken.

The current study supports the use of HRV as a measurement of emotional regulation in virtual environments and future studies should build on this finding by incorporating HRV as a manipulation check for emotional stimuli in VR protocols. Moreover, increased study of HRV in various induced-emotional states regardless of inducement means (e.g. VR, pictures, threat cues, etc.) is warranted in order to examine normal and abnormal ANS functioning within specific emotions. Finally, although support for the effectiveness of the mentor intervention was not demonstrated in the

present study, the limitations of the study and of the measurement of that intervention in particular indicate a need to refine, rather than to abandon the protocol. Further research to examine such refined protocol could assist in lowering the incidence of traumatic stress in service members and thereby reduce needless suffering.

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Table 1

Paired Samples t-tests for LF/HF ratio for Combat Scenarios compared to baseline for all participants

Event	*M(SD)*	*M* difference	*t*(38)
Baseline	0.79 (0.72)	-	-
Noise Startle	1.32 (1.39)	0.53	- 2.90**
CS1 Body	1.12 (0.83)	0.33	- 2.67*
CS1 Explosion	0.94 (0.67)	0.16	- 1.35
CS2 Explosion	1.12 (0.98)	0.33	- 2.11*
CS3 Beating	1.55 (1.14)	0.76	- 4.00***
CS4 Explosion	1.85 (1.85)	1.07	- 4.00***

Note. Mean Difference is amount above baseline. * $p < .05$ ** $p < .01$ *** $p < .001$

Table 2

Paired Samples t-tests for LF/HF ratio for Combat Scenarios 3 & 4 compared to second baseline for all participants

Event	*M(SD)*	*M* difference	*t*(38)
Baseline 2	0.77 (0.51)	-	-
Noise Startle 2	1.60 (1.08)	0.82	- 4.68***
CS3 Beating	1.55 (1.14)	0.77	- 4.05***
CS4 Explosion	1.85 (1.85)	1.08	- 3.52**

Note. Mean Difference is amount over baseline 2. ** $p = .001$ ***$p < .001$

Table 3

Paired Samples t-tests for LF/HF ratio comparison of all Combat Scenarios for all participants

Event	*M (M2)*	*M* difference	*t*(38)
CS1 Body – CS1 Exp	1.12 (0.94)	0.17	1.55
CS1 Body – CS2	1.12 (1.12)	- 0.004	- 0.03
CS1 Body – CS3	1.12 (1.55)	- 0.43	- 2.74**
CS1 Body – CS4	1.12 (1.85)	- 0.73	- 2.44*
CS1 Exp – CS2	0.94 (1.12)	- 0.18	- 1.27
CS1 Exp – CS3	0.94 (1.55)	- 0.61	- 3.82***
CS1 Exp – CS4	0.94 (1.85)	- 0.91	- 2.92**
CS2 – CS3	1.12 (1.55)	- 0.43	- 2.04*
CS2 – CS4	1.12 (1.85)	- 0.73	- 2.42*
CS3 – CS4	1.55 (1.85)	- 0.30	- 0.91

* $p < .05$ ** $p < .01$ *** $p < .001$

Table 4

Paired Samples t-tests for LF/HF ratio for Combat Scenarios compared to baseline for low baseline participants

Event	*M(SD)*	*M* difference	*t*(31)
Baseline	0.52 (0.26)	-	-
Noise Startle	0.97 (0.81)	0.45	- 3.05**
CS1 Body	0.94 (0.71)	0.42	- 3.21**
CS1 Explosion	0.78 (0.49)	0.26	- 2.92**
CS2 Explosion	1.01 (0.97)	0.50	- 2.77**
CS3 Beating	1.37 (1.11)	0.85	- 4.27***
CS4 Explosion	1.44 (1.55)	0.92	- 3.24**

Note. Low baseline = LF/HF baseline ratio < 1.0. Mean Difference is amount above baseline. $^{**}p < .01$ $^{***}p < .001$

Table 5

Paired Samples t-tests for LF/HF ratio for Combat Scenarios 3 & 4 compared to second baseline for low baseline participants

Event	*M(SD)*	*M* difference	*t*(31)
Baseline 2	0.78 (0.53)	-	-
Noise Startle 2	1.58 (1.04)	0.80	- 4.00***
CS3 Beating	1.37 (1.11)	0.60	- 3.04**
CS4 Explosion	1.44 (1.56)	0.67	- 2.267*

Note. Low baseline = LF/HF baseline ratio < 1.0. Mean Difference is amount over baseline 2. * $p < .05$ ** $p < .01$ *** $p < .001$

Table 6

Paired Samples t-tests for LF/HF ratio comparison of all Combat Scenarios for low baseline participants

Event	*M (M2)*	*M* difference	*t*(31)
CS1 Body – CS1 Exp	0.94 (0.77)	0.16	1.35
CS1 Body – CS2	0.94 (1.01)	- 0.07	- 0.43
CS1 Body – CS3	0.94 (1.37)	- 0.43	- 2.72*
CS1 Body – CS4	0.94 (1.44)	- 0.50	- 1.64
CS1 Exp – CS2	0.77 (1.01)	- 0.23	- 1.54
CS1 Exp – CS3	0.77 (1.37)	- 0.59	- 3.30**
CS1 Exp – CS4	0.77 (1.44)	- 0.66	- 2.21*
CS2 – CS3	1.01 (1.37)	- 0.36	- 1.55
CS2 – CS4	1.01 (1.44)	- 0.43	- 1.43
CS3 – CS4	1.37 (1.44)	- 0.07	- 0.21

Note. Low baseline = LF/HF baseline ratio < 1.0. * $p < .05$ ** $p < .01$

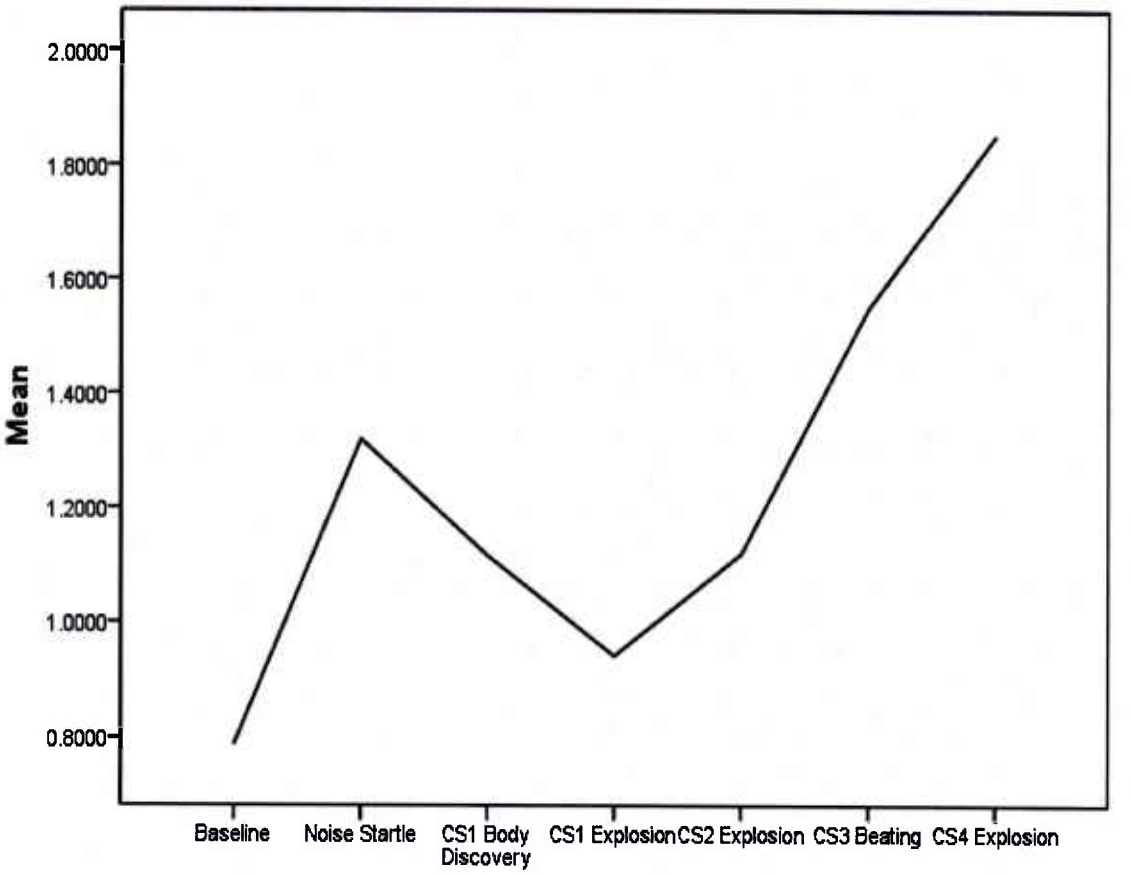

Figure 1. LF/HF ratios of Combat Scenarios for all participants.

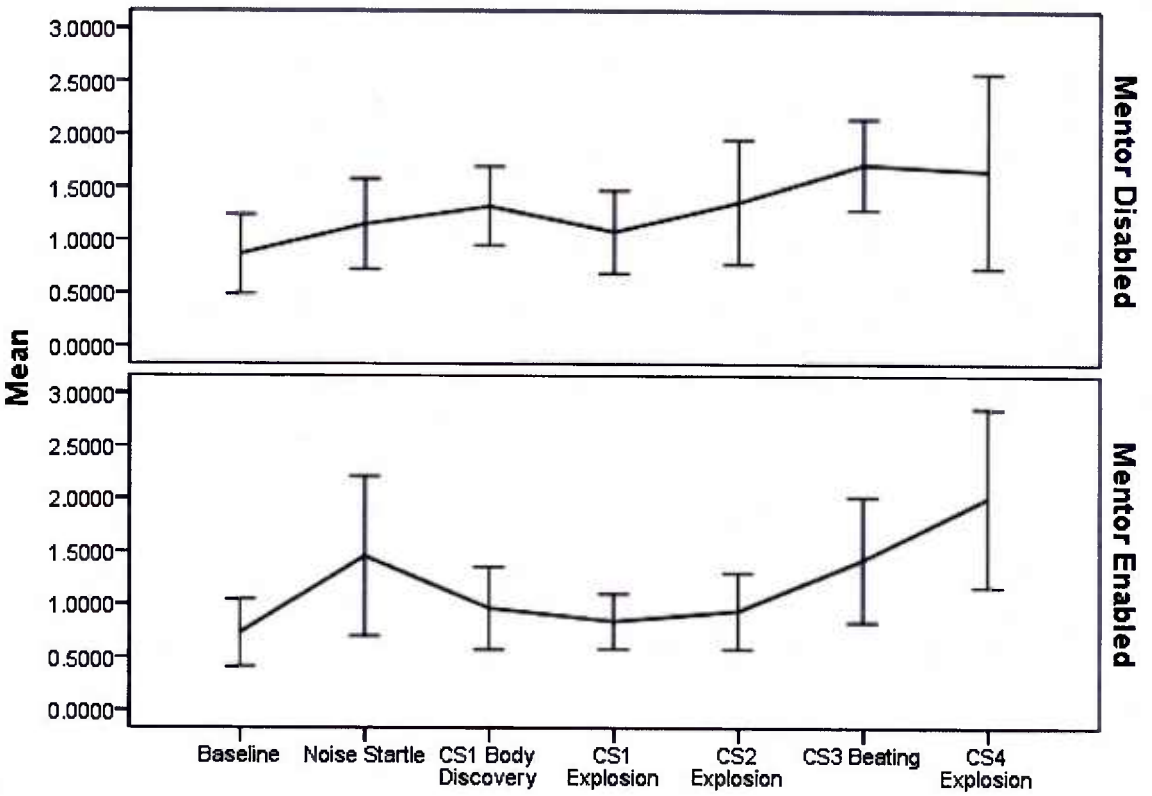

Figure 2. LF/HF ratios for Combat Scenarios by Mentor group for all participants.

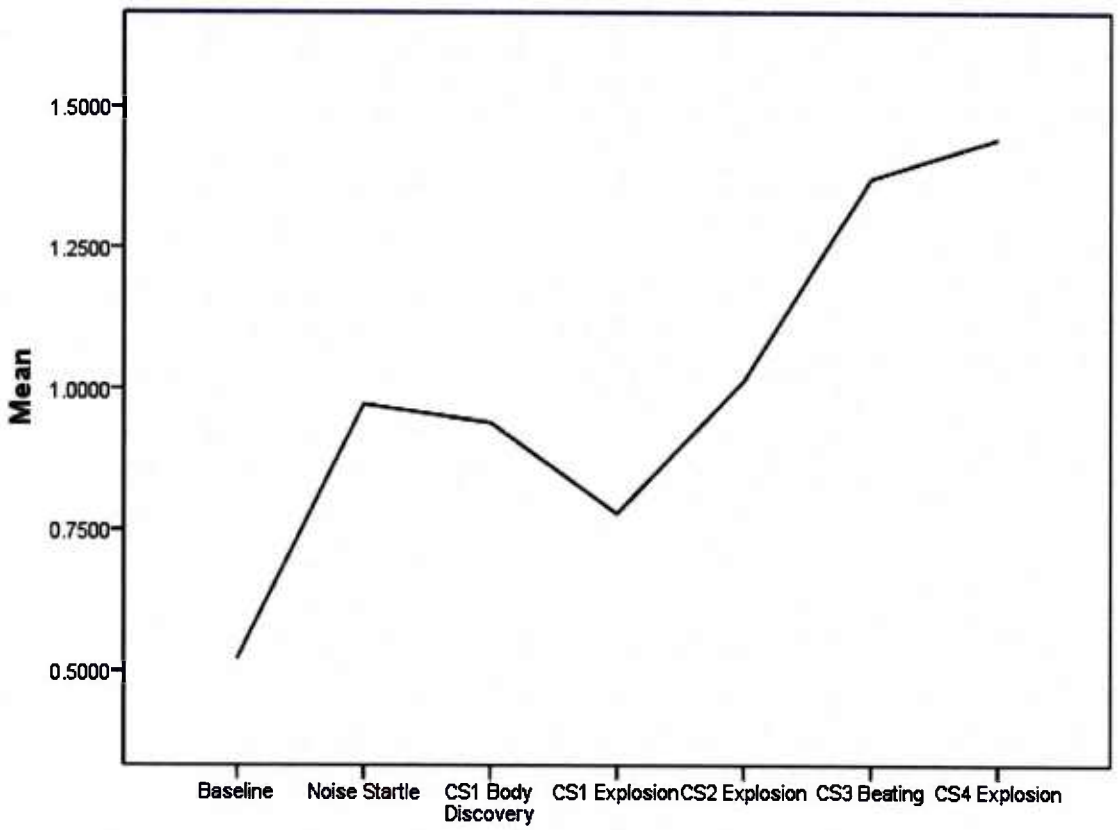

Figure 3. LF/HF ratios of Combat Scenarios for lower-baseline participants.

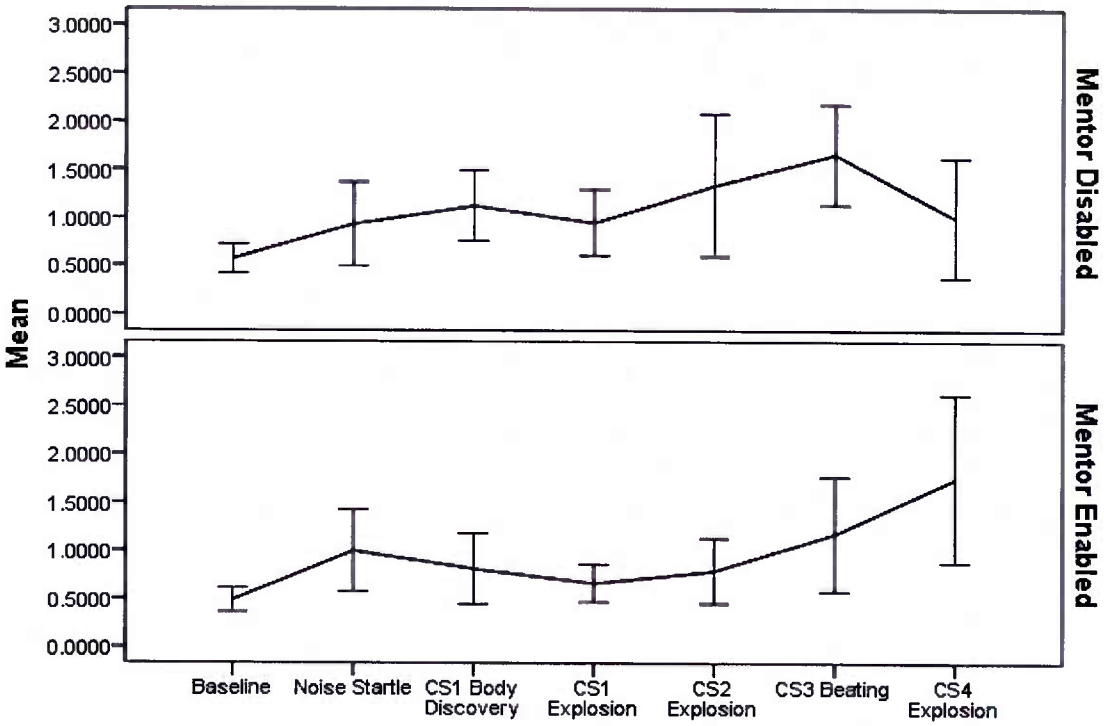

Figure 4. LF/HF ratios for Combat Scenarios by Mentor group for lower-baseline.

DHEA and Cortisol in Special Service Forces

Jared Rensberger, J. Galen Buckwalter, Albert A. Rizzo

In the face of trauma or extreme stress, a person typically responds in one of two ways: by developing pathological symptoms or by bouncing back relatively quickly without suffering adverse effects. Which response emerges depends on a web of interconnected factors including the person's physiological and psychological makeup (Feder, Nestler, & Charney 2009). Resilience can be broadly defined as a person's ability to bounce back from difficult experiences or intense stress (Yehuda, Flory, Southwick, & Charney, 2006), adapt amidst adversity or threatening situations, or maintain a stable equilibrium (Haglund, Nestadt, Cooper, Southwick, & Charney, 2007). Resilience is a dynamic, adaptive process that is changeable over time rather than a fixed construct or simply the lack of psychopathological symptoms (Russo, Murrough, Han, Charney, & Nestler, 2012). It is dependent on the interaction of various factors in the physiological and psychological domains (Lee et al., 2013). A resilient response to extreme stress or trauma is actually quite common (Bonanno, 2008). Individuals that possess a greater number of attributes associated with resilience are typically better equipped to adapt in the face of disruptive events (White, Driver, & Warren, 2010; Lee et al., 2013).

Some attributes that have been shown to correlate highly with resilience are: spirituality (Lee et al., 2013), adaptive engagement (Bonanno, 2008), sense of purpose (Rutten et al., 2013), and life satisfaction (Feder et al., 2009). There are also numerous physiological factors contributing to resilience (Karatoreos & McEwen, 2013), and the brain plays a key role in the physiological processes associated with a healthy response to stress. This study will explore the connection between resilience as a physiological process and a psychological construct.

HPA Axis

The impact of stress on the brain is mediated by the activation of the hypothalamic-pituitary-adrenal (HPA) axis, which consists of the stimulation of the paraventricular nucleus of the hypothalamus, resulting in the production of corticotropin releasing hormone (CRH) (Trickett, Noll, Susman, Shenk, & Putnam, 2010). This triggers the pituitary gland to release adrenocorticotropic hormone (ACTH) into the bloodstream. ACTH travels to the adrenal glands causing the synthesis of hormones that regulate the body's response to stress: dehydroepiandrosterone (DHEA) and cortisol (Charney, 2004). Inactivation of the HPA axis causes these systems to return to baseline levels of cortisol and DHEA, which typically happens after the threatening situation has passed (McEwen, 1998). The HPA axis is closely tied to both psychological and physical functioning, and disruption of the HPA axis can be problematic.

Cortisol

Cortisol is a corticosteroid hormone that promotes survival during dangerous situations by mobilizing the body's resources in case immediate action is necessary (Olff, Guzelcan, de Vries, Assies, & Gersons, 2006). In this way, it is responsible for the body's autonomic "fight or flight," physiological response when individuals are faced with anxiety provoking stimuli (McEwen & Gianaros, 2010). Increased cortisol release can also lead to increased energy, arousal, focused attention, fear memory formation, and fear learning (Haglund et al., 2007; Southwick, Vythilingam, & Charney, 2005). In the short-term, cortisol secretion is a critical component of the stress response system. However, prolonged exposure to cortisol can lead to depression, hypertension, osteoporosis, insulin resistance, abdominal obesity or coronary vascular disease (Southwick et al., 2005; Phillips et al., 2000). The relationship between HPA Axis functioning and resilience to extreme stress and trauma is still unclear. However, there is

evidence that the disruption of normal HPA functioning can lead to symptoms of depression and anxiety (Russo et al., 2012).

DHEA

Dehydroepiandrosterone (DHEA) is an endogenous hormone that is secreted concurrently with cortisol from the adrenal cortex in response to stress (Olff, Vries, Guzelcan, Assies, & Gersons, 2007). The most prevalent form of DHEA in the body is its sulfate ester, DHEA-S (Haglund et al., 2007). DHEA and DHEA-S, together called DHEA(S), have antiglucocorticoid effects on the brain, countering the deleterious effects of cortisol and serving as a neuroprotectant (Olff et al., 2006). Levels of DHEA(S) have been shown to increase under extreme stress (Russo et al., 2012), which is positively correlated with an increased ability to perform well (Yehuda et al., 2006). Increased levels of DHEA(S) have been positively associated with Posttraumatic Stress Disorder (PTSD) symptom improvement (Yehuda et al., 2006), implying that DHEA(S) levels may play a role in the recovery of PTSD. DHEA administration has been shown to decrease symptoms of depression and anxiety when administered to patients with major depression (Southwick et al., 2005). Additionally, there is a positive association between DHEA(S) and general well being (Rasmusson et al., 2004). These findings suggest that DHEA(S) may play an important role in modulating and adapting to the effects of stress on a physiological level.

Ratio of DHEA to Cortisol.

While cortisol and DHEA are both released concurrently in response to extreme stress and have different effects on the body, it is difficult to interpret their impact separate from one another. This is largely due to the change in hormonal levels across the lifespan. The production of DHEA declines as the person ages, a process known as adrenopause (Philips et al., 2010), but

cortisol remains stable across the lifespan (Philips et al., 2010). Yehuda et al. (2006) propose that considering the ratio of DHEA to cortisol may be a helpful method of determining the role these hormones play in the context of stress and trauma. A higher DHEA-to-cortisol ratio is associated with improved performance in military survival training and fewer dissociative symptoms following stress (Russo et al., 2012). Similarly, higher DHEA levels have been shown to be associated with PTSD symptom improvement (Feder et al., 2009). Higher DHEA-to-cortisol ratios may therefore indicate higher resilience to stress.

Hypotheses

This study aims to explore the connection between certain factors of psychological resilience and the underlying physiological processes in hopes of providing a clearer picture of how HPA Axis regulation and dysregulation can affect overall resilience. Spirituality, adaptive engagement, sense of purpose, and life satisfaction will be examined in correlation with DHEA(S), cortisol and the ratio of DHEA(S) to cortisol. There are four hypotheses proposed. First, a higher ratio of DHEA to cortisol would predict higher levels of spirituality. Second, a higher ratio of DHEA to cortisol would predict higher levels of adaptive engagement. Third, a higher ratio of DHEA to cortisol would predict an increased sense of purpose. The final hypothesis is that a higher ratio of DHEA to cortisol would predict increased levels of life satisfaction.

Method

Participants

A total of 28 members of the Army National Guard Special Forces were recruited via email to complete the Headington Institute Resilience Inventory (HIRI) and have biological specimens drawn. They were recruited voluntarily and gave written consent while not in the

presence of anyone but the researcher. All 28 (100%) participants completed the HIRI, and 27 of the 28 (96.43%) had biological specimens drawn. The mean age of the group is 41.43 (±8.185; 26-56). 26 of the 28 (92.6%) participants are male. Twenty three participants (82.2%) reported themselves to be White/Caucasian, 1 reported African American (3.6%), 2 reported Hispanic (7.1%), and 2 reported Asian (7.1%). 6 participants (21.4%) reported having 'some college' education, 2 reported they had a '2-year college degree' (7.1%), 9 reported they had a '4-year college degree' (32.2%), 8 reported they had a 'master's degree' (28.6%), 1 reported they had a 'doctoral degree' (3.6%), and 2 reported they had a 'professional degree (e.g. JD, MD)' (7.1%). The data for the present study were drawn from a larger study on stress resilience in virtual environments.

Procedure

A computer-based questionnaire was administered to members of the Army National Guard Special Forces. Before taking part in the study, participants were informed that participation was completely voluntary and were asked to provide informed consent. Participants were told that their responses would be confidential. They were informed that they would be rating a series of statements about certain behaviors and characteristics to see how well each one described their current state. They were also informed that they would be providing demographic and background information. Participants were cautioned that some of the prompts would include questions about traumatic events that they may have experienced. Participants were given contact information in order to ask questions or express concerns related to the study.

Biomarkers. Participants' blood and saliva were drawn to measure levels of select biomarkers. Dehydroepiandrosterone Sulfate (DHEA-S) from was drawn from 27 of 28 (96.3) participants' blood samples, measured as Micrograms per Deciliter (mcg/dL).

Dehydroepiandrosterone (DHEA) was drawn from 27 of 28 (96.3) participants' blood samples, measured as Nanograms per Deciliter. Cortisol was extracted from 23 of 25 (92%) participants' saliva samples and measured as mcg/dL.

Measures

Resilience. The Headington Resilience Inventory (HIRI; Nolty, Bosch, An, & Buckwalter, 2014) was administered to measure resilience, the participants' ability to adapt and recover in the face adversity over the life span. The HIRI includes 79 items that constitute 11 subsections. Participants rated themselves on a seven point Likert scale (e.g. 1 = *Not at all*; 4 = *Somewhat*; 7 = *Very well*). For the purpose of the present study, only four of the 11 subsections will be analyzed: spirituality (e.g., "My life is enriched by my spiritual beliefs, practices and/or experiences," $\alpha = .92$), adaptive engagement (e.g., "I can adapt to changing circumstances," $\alpha = .86$), sense of purpose (e.g., "I am not good at achieving long term goals," $\alpha = .81$), life satisfaction (e.g., "I am luck to be able to do the work I do," $\alpha = .77$). In the original HIRI study (Nolty et al., 2014), all correlations with the CD-RISC were statistically significant, ranging from $r = .25$ for Spirituality, to $r = .78$ for Adaptive Engagement.

Results

Participants

Of the 28 participants who participated in the study, all 28 completed the Adaptive Engagement, Spirituality, Sense of Purpose and Life Satisfaction subscales of the HIRI. There were 27 that had biological specimens drawn. Of these, 23 participants had saliva cortisol levels measures and 27 participants had levels of DHEA(S) measured.

Partial Correlation Analyses

Gender and age were entered as covariates in the analyses because DHEA(S) levels have been found to decline with age (Philips et al., 2010) and because gender differences in cortisol (Masi, Rickett, Hawkley, & Cacioppo, 2004) and DHEA(S) levels have been noted (Neigh, Gillespie, & Nemeroff, 2009). Cortisol and DHEA(S) levels were standardized using a z-score conversion, and a log transformation was utilized to correct for skewness. A One-Way ANOVA revealed that there were not significant gender differences in the ratio of DHEA to cortisol $F(1, 22) = 1.72$, $p = .20$, or the ratio of DHEA-S to cortisol, $F(1, 22) = .44$, $p = .51$. Similarly, there were not significant gender differences in levels of DHEA-S, $F(1, 26) = .12$, $p = .73$, or cortisol, $F(1, 22) = .14$, $p = .71$. However, it was found that female participants had significantly higher levels of DHEA than male participants, $F(1, 26) = 5.2$, $p = .03$. A bivariate correlation revealed that there were not significant associations between age and levels of cortisol $r(23) = .21$, $p = .34$, the ratio of DHEA to cortisol, $r(23) = -.09$, $p = .70$, or the ratio of DHEA-S to cortisol, $r(23) = .11$, $p = .62$. However, age was significantly correlated with levels of DHEA, $r(27) = -.44$, $p = .02$, and levels of DHEA-S, $r(27) = -.49$, $p = .01$.

Contrary to the first hypothesis, with age and gender as covariates, levels of adaptive engagement were not associated with the ratio of DHEA to cortisol, $r(19) = -.01$, $p = .97$, or the ratio of DHEA-S to cortisol, $r(19) = -.04$, $p = .88$. In support of the second hypothesis, we found that with age and gender controlled, levels of spirituality were associated the ratio of DHEA to cortisol, $r(19) = .56$, $p = .009$, and the ratio of DHEA-S to cortisol, $r(19) = .49$, $p = .025$. Contrary to the third hypothesis, we found that after controlling for effects of age and gender, sense of purpose was not associated with the ratio of DHEA to cortisol, $r(19) = -.08$, $p = .73$ or the ratio of DHEA-S to cortisol, $r(19) = .002$, $p = .99$. Contrary to the fourth hypothesis, we

found that with age and gender controlled, life satisfaction was not associated with the ratio of DHEA to cortisol $r(19) = -.19$, $p = .41$ or the ratio of DHEA-S to cortisol, $r(19) = -.07$, $p = .75$.

Discussion

The body's secretion of stress hormones is thought to be closely connected with psychological functioning. This study explored the link between stress hormones and aspects of psychological resilience. More specifically, this study focused on DHEA and cortisol, the two primary stress hormones released by the HPA axis in response to intense stress, and their relationship to multiple aspects of resilience as measured by the HIRI. The ratio of DHEA to cortisol was used as a predictor rather than looking at them in isolation given the level of change these hormones can be subject to across the life span (Yehuda et al., 2006). Spirituality was significantly correlated with an increased ratio of DHEA to cortisol. However, there were multiple facets of resilience including adaptive engagement, sense of purpose, and life satisfaction that showed no significant relationship to the ratio of DHEA to cortisol.

A major goal of the study was to explore the relationship between soldiers' reported level of spirituality and the amount of stress hormones present in the body. It was predicted that as levels of spirituality increased, the ratio of DHEA to cortisol would increase as well. Spirituality was found to be significantly correlated with the ratio of DHEA to cortisol. These findings complement the work of Seeman et al. (2006) who found that transcendent meditation increased a person's ratio of DHEA-S to cortisol. This suggests that as spirituality becomes more important in the lives of these Special Forces members, they are more likely to have an altered physiology and an increased level of physiological resilience. These findings highlight the positive impact that spiritual beliefs and practices can have on soldiers as they prepare for combat and potentially traumatic experiences.

This study also sought to examine the relationship between adaptive engagement and a person's ratio of DHEA to cortisol. Given the numerous positive outcomes that have been associated with an increase in this ratio, it was expected that as adaptive engagement increased, the ratio of DHEA to cortisol would increase as well. Contrary to expectations, no relationship was found between adaptive engagement and this ratio of stress hormones. This is surprising because adaptive engagement is thought to be highly correlated with resilience (Nolty et al., 2014). Similarly, there was not a significant relationship between a person's life satisfaction and a person's ratio of DHEA to cortisol. This was not expected because life satisfaction has been previously thought of as an indicator of resilience (Lee et al., 2013). In the same vein, sense of purpose in life was not associated with the ratio of DHEA to cortisol, which is surprising because sense of purpose is thought to be a building block for resilience (Rutten, 2013).

In light of these findings, it is unclear how stress hormones function in members of the Special Forces. In many ways, the participants from this study were thought to embody resilience to a high degree given their choice of profession and their intense training. It seems short sighted to declare that these participants are not resilience in spite of strong correlations for multiple aspects of resilience. One explanation for the lack of significant findings could be that the military training and combat experiences could disrupt normal HPA axis functioning. Yehuda (2006) notes that studies have shown various results in participants with PTSD in that the ratio of DHEA to cortisol has been anywhere from increased to decreased to neutral. It is possible that although these participants have not developed PTSD, they may suffer from a disruption in HPA axis functioning. This could lead to results that do not follow expectations due to potential attenuation or hypersuppression of cortisol levels (Trickett, Noll, Susman, Shenk, & Putnam, 2010).

It is also possible that the standard for what a resilience person looks like is different in the Special Forces than it is for the general public. One of the most important indicators of resilience is adaptive engagement, which can be described as a person's flexibility and ability to easily adapt to the situation. The rigid routine, training, and lifestyle of the Special Forces may not foster or value flexibility to the same degree as other professions like humanitarian aid workers. This could lead to a profile of resilience that looks somewhat different than what would be expected.

The results of this study should be considered in light of inherent limitations. The participants for this study are primarily male and they are all members of the Army National Guard Special Forces. This makes generalization difficult because the sample does not represent the population well. Additionally, this study did not consider the effects of other hormones such as Neuropeptide Y, which may affect the findings to some degree. Given that this studied relied on self-report measures, there may be a response bias and some defensive distortion particularly given that vulnerability is not likely emphasized in Special Forces training. Finally, inferences about causal effects cannot be drawn because of the cross-sectional nature of the study.

Given that this study only looked at levels of DHEA(S) and cortisol as biomarkers for resilience, future studies would do well to consider additional hormones and physiological data to strengthen the way that resilience is looked at and conceptualized on a physiological level. It may also be beneficial to compare these results with a more heterogeneous or civilian sample. Due to the effects of PTSD on levels of stress hormones (Trickett, et al., 2010), future research may also focus on examining the comparison between physiological and psychological resilience in a sample consisting of participants that have been diagnosed with PTSD.

In conclusion, as far as we can tell the data reported here are the first to directly examine the link between stress hormones and these particular aspects of resilience. These findings are notable given the high level of resilience training the participants have had as a part of their military experience. Although a causal relationship cannot be conferred, the results add to the view that psychological resilience is closely linked to the physiological functioning of the brain.

Table 1.

Demographic Information of Sample Population

	Percent	*M* (*SD*)
Age	-	41.4 (8.19)
Gender		
Male	92.6%	-
Female	7.1%	-
Education		
Some college education	21.4%	-
2-year college degree	7.1%	-
4-year college degree	32.2%	-
Master's degree	28.6%	-
Doctoral degree	3.6%	-
Professional degree (e.g. JD, MD)	7.1%	-
Ethnicity		
White/Caucasian	82.2%	-
African American	3.6%	-
Hispanic	7.1%	-
Asian	7.1%	-

Note. $N = 28$.

Table 2.

Bivariate Correlations between Main Study Variables

	DHEA	DHEA-S	Cortisol	DHEA/Cortisol	DHEA-S/Cortisol
Adaptive Engagement	.25	-.11	.30	.05	-.04
Life Satisfaction	.22	.23	.30	-.19	-.07
Spirituality	.013	-.07	.09	.56**	.49*
Sense of Purpose	.19	.20	.46*	-.08	.002

Notes. DHEA = dehydroepiandrosterone. DHEA-S = dehydroepiandrosterone sulfate.

*$p < .05$ **$p < .01$.

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Heart Rate Variability and Stress Resilience Among Active Duty National Guard Soldiers

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Although the concept of resilience is widely recognized, recent research has found that it is still not well understood due to its multifaceted nature. Resilience is known to regulate an individual's response to traumatic events and is described by Bonanno (2008) as the "ability of individuals to maintain relatively healthy levels of psychological and physical functioning" (p. 102) in adverse, traumatic events. However, resilience is not simply a recovery process in which an individual gradually returns to a baseline level of functioning; rather, resilience involves the ability to adapt to unplanned changes and to maintain a stable equilibrium even after experiencing a traumatic stressor (Bonanno & Mancini, 2012; Haglund, Nestadt, Cooper, Southwick, & Charney, 2007; Karatsoreos & McEwen, 2011; Luthar, Cicchetti, & Becker, 2000).

There are varying components of healthy resilience that have been explored and have emerged in the literature. For example, relational supports have been found to be important factors for resilience (Boscarino, 1996; Travis, Lyness, Shields, King, & Cox, 2004). Physiological factors such as maintenance of physical well-being (Bonanno, Galea, Bucciarelli, & Vlahov, 2007) and genetics (Gillespie, Phifer, Bradley, & Ressler, 2009) also play a role in conferring resilience. Also, utilization of adaptive coping and problem solving skills have been shown to be important pathways for resilience—dissociating from negative emotions and thoughts (Bonanno & Field, 2001; Weinberger, Schwartz, & Davidson, 1979), regulating emotion by using positive emotion and laughter (Fredrickson, 2001; Keltner & Bonanno, 1997; Tugade, Fredrickson, & Barrett, 2004), adapting to changing conditions (Rutter, 1985), employing self-efficacious behavior such as viewing negative experiences as opportunities to learn and grow (Kobasa, Maddi, & Kahn, 1982; Rutter, 1985), and practicing beneficial self-care activities (Rutter, 1985). In addition, possessing high self-confidence and self-esteem have been shown to be important elements of resilience (Bonanno, Field, Kovacevic, & Kaltman, 2002;

Bonanno, Rennicke, Dekel, & Rosen, 2003). In summary, multiple factors—biological, psychological, and relational—appear to contribute to one's resilience in the presence of adversity (Bonanno, 2008; Luthar, Doernberger, & Zigler, 1993; Rutter, 1999).

Because there are many frameworks from which to understand resilience, there has been much difficulty among researchers to come to a consensus regarding the conceptualization, operationalization, and assessment of the construct of resilience (Luthar et al., 2000). Defining or obtaining a solid theoretical understanding of the construct and the mechanisms by which resilience works becomes a complex task because of the multidimensional nature of resilience. However, recent discoveries made in the field of neurophysiology and psychophysiology have made it possible to develop a more concrete understanding of the mechanisms behind resilience. In this study, we will investigate a potential biomarker of stress resilience called heart rate variability.

Heart Rate Variability

For the past four decades, heart rate variability (HRV) has received much popular attention given its valuable utility in measuring health outcomes. HRV initially gained clinical significance when shown to be a reliable, independent predictor of mortality following an acute myocardial infarction (Bigger, Fleiss, Rolnitzky, & Steinman, 1992; Kleiger, Miller, Bigger, & Moss, 1987). Now, HRV changes, usually lowered HRV, have been found to be a risk factor for a wide range of pathophysiology and psychopathology (Berntson et al., 1996; Dekker et al., 2000; Di Simplicio et al., 2011; Mujica-Parodi et al., 2009; Shaikh al arab et al., 2012). As more experimental research findings have discovered a high correlation between cardiovascular mortality and the autonomic nervous system (i.e., increased sympathetic or reduced vagal activity), quantitative measures of autonomic activity have gained more clinical attention (Task

Force, 1996). HRV remains to be one of the most promising measures of autonomic health given its popularized, easy-to-use and accessible method. HRV continues to gain wide support for its potential to provide insight into pathological conditions and health risk reduction.

Underlying physiology of HRV. Although there are numerous physiological and environmental factors that can influence HRV, with regard to psychophysiology, the most relevant influence is the autonomic nervous system. HRV reflects how well an individual can modulate his or her cardiac activity to meet situational demands and stressors that can trigger emotional and physiological arousal. The autonomic nervous system plays a key role in the generation of these emotions and accompanying varying degrees of physiological arousal. This system is subdivided into the excitatory sympathetic nervous system and inhibitory parasympathetic nervous system, each having differential effects. For instance, during times of experiencing physical or psychological stress, the sympathetic nervous system plays a dominant role and induces physiological arousal (e.g., increased heart rate) to facilitate adaptation to a stressor. On the contrary, during periods of rest and relatively safety, the parasympathetic nervous system takes over in playing the dominant role and maintains a lower degree of physiological arousal (e.g., decreased, resting heart rate). Sympathetic and parasympathetic systems have antagonistic effects on cardiac activity. For example, an increased sympathetic activity or decreased parasympathetic activity results in an increased heart rate. Moreover, a flexible autonomic nervous system allows an individual to transition between high and low arousal states with ease; that is, the individual can rapidly modulate physiological and emotional arousals that are elicited by environmental stressors (Appelhans & Luecken, 2006). The autonomic nervous system's ability to quickly vary heart rate allows for effective and appropriate adjustment to environmental stressors. Conversely, an individual with autonomic

rigidity is less capable in effectively regulating physiological and emotional arousal in accordance with environmental stressors. In sum, HRV is essentially a measure of the constant interaction between the sympathetic and parasympathetic effects on heart rate, which also provides insight about autonomic flexibility—the capacity for effective regulation of stress-related emotional and physiological arousal.

Regarding the mechanism of effect, both the sympathetic and parasympathetic nervous systems affect heart rate by influencing the sinoatrial node, which is the heart's primary pacemaker. Sympathetic activation has an excitatory effect on the sinoatrial node, which increases heart rate. Sympathetic activity is mediated by the neurotransmitter, norepinephrine, which acts slowly on cardiac activity—peak effect occurring approximately four seconds and returning to baseline in about 20 seconds. On the other hand, parasympathetic activation has an inhibitory effect and decreases heart rate via a much faster acting neurotransmitter called acetylcholine—peak effect occurring approximately 0.5 seconds and returning to baseline in about one second (Berntson et al., 1997; Pumprla, Howorka, Groves, Chester, & Nolan, 2002). In addition, the parasympathetic nervous system affects cardiac activity via the vagus nerve; hence, parasympathetic activity is also referred to as vagal activity. Although both branches of the autonomic nervous system constantly influence heart rate, parasympathetic activity has greater influence at rest and serves to maintain a baseline, resting firing rate (Berntson et al., 1997). Furthermore, the two autonomic branches have different latencies of action; therefore, produce oscillations in heart rate at different frequencies. This difference serves as the basis for there being variability in heart rate.

Respiration has also been found to influence HRV. Inhalation suppresses parasympathetic influence on heart rate, which produces an increase in heart rate (Berntson,

Cacioppo, & Quigley, 1993). However, exhalation reactivates parasympathetic influence on heart rate, which results in a decrease in heart rate. This rhythmic oscillation in heart rate influenced by respiration is known as a phenomenon called respiratory sinus arrhythmia (RSA; Bernardi, Porta, Gabutti, Spicuzza, & Sleight, 2001; Berntson et al., 1993). RSA has been found to produce a large aspect of parasympathetic mediated variation in heart rate (Berntson et al., 1997). Although there has been some debate about RSA being an index of vagus nerve activity (Pyetan & Akselrod, 2003), many researchers have argued that RSA is an index of HRV that is mediated by the parasympathetic nervous sytem.

Psychophysiological theories of HRV. There are two major theories regarding the causal relationship between autonomic flexibility, including HRV and its neuroanatomical substrates, and emotional regulation. One is the polyvagal theory which is based on an evolutionary perspective (Porges, 1995, 2001). This theory posits that the autonomic nervous system structures evolved through three stages, and each stage's corresponding structure has distinct functions in regulating social and adaptive coping behaviors. The other is the neurovisceral integration theory which conceptualizes the relationship between emotional regulation and HRV from a dynamical systems perspective (Thayer & Lane, 2000)

Polyvagal theory. Porges' (1995, 2001) theorized that autonomic activity and behaviors like emotional regulation are related. He postulated that the human ANS evolved through three, phylogenetic stages. Each stage is associated with a distinct autonomic subsystem, which continues to be retained and expressed via social processes and specific adaptive behaviors in response to environmental signs of safety, danger, or threat. The first stage involved the acquisition of the dorsal vagal complex within the brainstem, which is a primitive, slow-responding, unmyelinated vagus nerve. This ancient vagus nerve has a depressive function—

slows heart rate by inhibiting the cardiac sinoatrial node, in order to conserve metabolic resources and support vegetative processes. Furthermore, the dorsal vagus supports threat response strategies associated with immobilization behaviors (e.g., freeze response, passive response, feigning death). The second stage involved the development of the sympathetic system which supports threat response strategies associated with active mobilization behaviors (e.g., fight or flight). The third and final stage involved the evolution of the ventral vagus within the brainstem, which is only observed in mammals.

Unlike the dorsal vagus, the ventral vagus is myelinated, flexible, and fast-acting, such that it works to quickly activate or suppress its inhibitory influence on the cardiac sinoatrial node. Also, this newer vagal circuit is linked to cranial nerves that regulate the muscles of the face and head, which mediate social engagement behaviors (e.g., facial expression, nonverbal communication, vocalization, listening; Porges, 2001). These various neuroanatomical connections (e.g., "face-heart connection") provide a mechanism for the interaction between emotional states underlying social behaviors and cardiac activity (Porges, 1995). For instance, the face-heart connection allows humans to use vocal prosody and facial expression to convey their physiological state to others (Stewart et al., 2013). Moreover, the ventral vagus supports threat regulation by fostering calm behavioral states via inhibiting sympathetic influences to the heart and dampening stress response pathways (e.g., hypothalamic-pituitary-adrenal axis; Bueno et al., 1989; Porges, 2009). In addition, via the face-heart connection, the ventral vagus further supports threat regulation through use of social engagement behaviors and social communication to regulate one's physiological state. For example, when safety is communicated via social behaviors (e.g., nonverbal language), defensiveness and physiological arousal is down-regulated (Geller & Porges, 2014). In sum, the polyvagal theory emphasizes a hierarchical relation among

three autonomic subsystems that evolved to support adaptive responses. The human autonomic response strategy to stressors starts with the newest structure of the evolutionary hierarchy; however, the other autonomic subsystems activate if the ventral vagal complex's withdrawal response is insufficient to meet situational demands.

Neurovisceral integration theory. Thayer and Lane (2000, 2009) proposed a model based on a dynamical systems perspective to conceptualize the relationship between emotional regulation and HRV. Within this framework, it is understood that many external and internal sources (e.g., physiological, behavioral, emotional, cognitive, social, environmental) influence how individuals adapt to environmental stressors. Given the diversity of such influences, successful adaptation is then determined by how flexibly one can adjust to changing situational demands. Moreover, this model claims that a core set of neural structures, the *central autonomic network*, provides individuals with the ability to integrate the various external and internal influences, and to adaptively regulate interacting subsystems (i.e., perception, cognition, behavior, physiology) that are responsible for producing specific emotional states (Hagemann, Waldstein, & Thayer, 2003; Thayer & Lane, 2000). For instance, this neural system continually evaluates the level of safety and threat in the environment in order to prepare the individual to take appropriate action; this neural system also works to complement an individual's internal homeostatic processes to the external environment in order to help the individual make appropriate, adaptive adjustments.

The extensive neural network making up the central autonomic network, remotely regulates autonomic influences on heart rate (Benarroch, 1993). This network includes several neural structures, including cortical (prefrontal and insular cortices), limbic (anterior cingulate, hypothalamus, amygdala), and brainstem (periaqueductal grey, ventrolateral medulla, nucleus of

the solitary tract) regions. These neural correlates are all reciprocally interconnected, where there is bidirectional transmission of information (Benarroch, 1993). The central autonomic network receives afferent inputs from visceral organs regarding internal, homeostatic conditions, as well as from sensory processing areas regarding the external environment. These inputs allow this network to flexibly adjust physiological and emotional arousal in response to both internal and external conditions. The central autonomic network also transmits outputs to various internal organs. However, the primary output of this network, mediated by the SNS and PNS (i.e., vagal nerve), is transmitted to the cardiac sinoatrial node. In addition, the output of the central autonomic network is controlled by tonic inhibition via parasympathetic influences. The interplay of outputs to the heart produces variability in heart rate. Thus, HRV is a measure of central autonomic network outputs, and by proxy, reflects an individual's ability to regulate physiological and emotional responses (Thayer & Lane, 2000; Thayer & Siegle, 2002). Furthermore, the central autonomic network represents a critical component of the brain network that controls visceromotor, neuroendocrine, and behavioral responses that are required for adaptive, goal-directed behavior and homeostatic regulation.

HRV may provide an index of the degree to which the central autonomic network supports emotion regulation and flexible, adaptive adjustment to changing situational demands. With regard to physiological regulation, specifically regulation of the heart, a balanced system is considered healthy because the system can respond to a wide variety of physiological and environmental demands (Thayer and Sternberg, 2006). However, a system that is rigid and fixated to a particular pattern is considered dysregulated. Therefore, a heart rate of a healthy, balanced heart oscillates spontaneously (i.e., high HRV), whereas a diseased, dysregulated heart has little to no HRV. HRV may serve as an easily measured output of the central autonomic

network, which may also provide important information regarding an individual's ability to effectively function and adapt in a stressful, changing environment.

Comparison between the polyvagal theory and neurovisceral integration theory. Taken together, both theories appear to be more complementary than oppositional (Appelhans & Luecken, 2006). For instance, both theories emphasize the integral role of parasympathetic activity in inhibiting autonomic arousal during emotional regulation. In addition, both theories argue that HRV is an invaluable measure of an individual's capacity for emotional regulation. Regarding differences, other than having different theoretical frameworks (e.g., evolutionary perspective versus dynamical systems perspective), each theory also has a unique neuroanatomical emphasis. Although the neurovisceral integration theory argues for neuroanatomical links between the autonomic nervous system and certain brain regions related to emotional processing, the polyvagal theory largely focuses on the neutral connections between the vagal nerve and other cranial nerves. These differences regarding neuroanatomical correlates have led to diverging implications. For instance, studies related to the neurovisceral integration model have focused more heavily on emotional dysfunction, whereas studies related to the polyvagal theory have focused more on social processes.

Measurement of HRV. HRV is measured by calculating the variation among a set of temporally ordered inter-beat intervals from a continuous measure of heart rate. The most basic component of HRV measurement is estimating the time between one beat to the next, which is generally referred to as the *normal-to-normal* intervals. In HRV analysis, inter-beat intervals are usually defined as the temporal distance between R-R spikes found on an electrocardiogram (ECG), a test that records cardiac electrical activity. There are many ways to measure HRV, but proper measurements have been delineated by a leading group of researchers in the field (Task

Force, 1996). For instance, HRV can be evaluated in three ways—statistical, frequency, or geometric analyses. For the purpose of this study, geometric analyses will not be discussed given that they require more extended recordings of ECG and also provide less accurate estimates of HRV (Task Force, 1996).

Statistical analyses. For these types of analyses, variance-based calculations are performed on a set of normal-to-normal intervals, yielding estimates for HRV in temporal units (e.g., milliseconds). One commonly used statistical analysis is the standard deviation of the normal-to-normal intervals interval. Another commonly used analysis is the standard deviation of the average normal-to-normal interval calculated across all five minute segments of an ECG recording. Although using the standard deviation of normal-to-normal intervals represents the overall view of HRV, this statistic represents HRV occurring at cycles longer than five minutes. Another popular analysis is the square root of the mean squared difference of successive normal-to-normal intervals. This statistic has been found to be a useful estimate of shorter HRV segments (Task Force, 1996). Furthermore, one major limitation of statistical analyses is that large amounts of ECG recording time (i.e., 24 hours) are recommended (Task Force, 1996). Therefore, whereas statistical analyses are well-used in medical studies where subjects tend to be monitored for longer lengths of time, these analyses are ill-suited for psychophysiological research which tend to gravitate towards using much shorter recording times.

Frequency analyses. A relatively new and refined technique, among the frequency class of analyses, is called power spectral analysis. Power spectral analysis has been more commonly used in psychophysiological literature given that this analysis requires only nominal recording time of five minutes or even shorter. This analysis partitions the variance of the normal-to-normal interval into a power spectrum, which shows a distribution of HRV occurring at different

frequencies. Among the various approaches used to extract various frequency components from a normal-to-normal interval, the most common is the Fast Fourier Transformation (Appelhans & Luecken, 2006). Transforming the ECG data this way results in a power spectrum which reveals a bimodal distribution with peaks in high frequency (HF) and low frequency (LF) ranges. The nominal range for HF is 0.15-0.40 Hz, which also is the normal frequency of adult respiration (Task Force, 1996). Moreover, the HF range has also been widely accepted as vagal influence on HRV. On the other hand, the nominal range for LF is 0.04-0.15 Hz (Task Force, 1996). Unlike the widely-accepted understanding for the physiological nature of HF HRV, much debate exists regarding the exact nature of LF HRV. Some have argued that LF can reflect sympathetic activity. Others have contended for the use of the very low frequency (VLF), which is less than 0.04 Hz, as a better marker of sympathetic activity (Task Force, 1996). However, use of VLF still remains to be controversial given the lack of supporting psychophysiological research.

Low frequency HRV controversy. Researchers have found that HF HRV and LF HRV is a direct marker of parasympathetic and sympathetic activity, respectfully (Malliani, Pagani, Lombardi, & Cerutti, 1991; Pagani et al.,1986; Wheat and Larkin, 2010); in addition, the ratio of LF to HF components of HRV has been found to be a useful measure of *sympathovagal balance* between the sympathetic and parasympathetic influences on the heart (Malliani, Pagani, Lombardi, and Cerutti 1991). Although the Task Force (1996) have also conceded to similar views, they have also argued that LF HRV is actually a reflection of both sympathetic and parasympathetic activity. Moreover, the LF/HF ratio has been theorized to represent the constant competition between sympathetic and parasympathetic control of the central autonomic network (Appelhans & Luecken, 2006). On the other hand, some researchers have come to opposing conclusions (Eckberg, 1997). Based on studies where experimentally blocking or inducing

sympathetic activity did not result in expected changes (i.e., reduce or increase LF power, respectfully), some researchers have posited that LF HRV only reflects parasympathetic activity rather than sympathetic activity (Reyes Del Paso, Langewitz, Mulder, van Roon, & Duschek, 2013). These same researchers have also disregarded the meaningfulness of the LF/HF ratio, stating that there was no validity in assuming the sympathetic and parasympathetic branches act in a concerted, balanced effort. Despite the controversy that exists regarding the exact nature of LF, HF, and their ratio, there is still much agreement among researchers for the positive utility of spectral power analysis as a non-invasive measurement of autonomic flexibility. Many prominent researchers still continue to use LF HRV as a measure of sympathetic activity and LF/HF ratio as a measure of autonomic balance (Reyes Del Paso et al., 2013).

Some researchers have argued that both HF and LF HRV are markers of parasympathetic activity, but also acknowledged that HF and LF are different given their weak correlation together when compared with other markers of cardiac activity (Reyes Del Paso et al., 2013). In light of this ambiguity, Porges' (1995, 2001) polyvagal theory may shed further clarity on the nature of the various HRV variables. According to the polyvagal theory, it is possible that both HF and LF are markers of vagal activity, as argued by some researchers. However, to be more precise, HF HRV may in fact reflect the increased inhibitory activity of the ancient, dorsal vagus, whereas the LF HRV may reflect the increased inhibitory activity of the newer, ventral vagus. This trajectory of thinking aligns with some studies that have found that pharmacological blockage of the vagus nerve resulted in an overall decrease of the HRV power spectrum and an increase of the LF/HF ratio (Reyes Del Paso et al., 2013). Moreover, if LF HRV is indeed a measure of ventral vagal activity, it would be comprehendible that LF HRV would increase along with an increase in sympathetic activity. Furthermore, LF HRV may be seen as a proxy

measurement of increased sympathetic activity.

Psychophysiological Literature using HRV

HRV in psychopathology. Low HRV has been associated with several psychological disorders, including anxiety disorders (McCraty, Atkinson, Tomasino, & Stuppy, 2001) and depression (Carney et al., 2000; Rechlin, Weis, Spitzer, & Kaschka, 1994). There have also been several studies showing that low HRV is related to mental health disorders related to disruptions of autonomic function, such as Post-traumatic Stress Disorder (PTSD; Cohen et al., 2000; Lakusic, 2007; Lee & Theus, 2012). One major cluster of symptoms associated with PTSD is hyperarousal (e.g., irritability, hypervigilance, difficulty concentrating, being easily startled, difficulty falling or staying asleep), which suggests dysregulation within the autonomic nervous system, specifically attenuated parasympathetic activity and elevated sympathetic activity (Blechert, Michael, Grossman, Lajtman, & Wilhem, 2007). In an earlier study exploring HRV changes during stress exposure, researchers discovered that whereas control subjects displayed significant autonomic activity when recounting a traumatic event, PTSD patients demonstrated almost no autonomic activity (Cohen et al., 1998). However, the researchers found that PTSD patients at rest demonstrated a degree of autonomic hyperactivity (i.e., higher HRV LF) that was comparable to control subjects who were in active engagement with stressors. These findings suggested that PTSD patients were unable to deploy further autonomic responses to stressors given their inordinate autonomic activity just at rest. In another similar study, at rest, PTSD patients and patients with panic disorder had significantly higher HRV LF and lower HRV HF on power spectrum analyses (suggesting increased sympathetic activity) when compared to control subjects (Cohen et al., 2000). However, unlike patients with panic disorder and control subjects, PTSD patients did not exhibit any significant differences in LF or HF HRV during

active engagement with stressors. This study, along with others, further supports that chronic autonomic dysregulation—overstimulated sympathetic activity and decreased parasympathetic activity—is a possible mechanism that maintains PTSD (Blechert et al., 2007; Milliken, Auchterlonie, & Hoge, 2007).

HRV implications for treatment. Given that HRV is a simple, noninvasive indicator of autonomic activity, there is great potential for using HRV biofeedback as a therapeutic intervention for mental health conditions that involve dysregulation of the autonomic nervous system. Biofeedback interventions use various instruments that provide individuals with instant, accurate feedback of physiological function (e.g., HRV, respiration, muscle tension). The goal is to help individuals learn how to manipulate these functions in order to improve health and performance outcomes. A specific HRV biofeedback technique that helps individuals to control and increase HRV is called respiratory sinus arrhythmia (RSA) biofeedback. RSA biofeedback aims to train individuals in *resonance* frequency breathing (Vaschillo, Lehrer, Rishe, & Konstantinov, 2002). Resonance frequency in HRV is defined as the frequency at which maximum HRV is produced, approximately 0.1 Hz for most individuals (Lehrer, Vaschillo, & Vaschillo, 2000). RSA biofeedback utilizes respiratory sinus arrhythmia (i.e., respiratory-induced heart rate oscillations). By leading individuals to breathe very slowly—approximately six breaths per minute—individuals can reach resonance frequency. Moreover, this frequency range also happens to correspond to cardiac rhythms caused by baroreflex activity. The baroreflex is a homeostatic reflex that modulates blood pressure via changing heart rate (e.g., when blood pressure increases, this system reduces heart rate via the parasympathetic nervous system; Vaschillo, Vaschillo, & Lehrer, 2006). Furthermore, when individuals breathe at rates corresponding to baroreflex effects, *resonance* occurs—the two sources of heart rate variability

(e.g., RSA and baroreflex activity) interact, producing very high amplitudes at a single frequency and accounting for higher total HRV (Lehrer et al., 2003). Continued breathing at one's resonance frequency stimulates and increases the efficiency of the baroreflex system, which produces a number of benefits such as strengthening indirect modulation of autonomic activity (Lehrer et al, 2003). In sum, the goal of RSA biofeedback is to stimulate both RSA and the baroreflex, which ultimately produces resonance properties of the cardiovascular system—larger HRV and large-amplitude stimulation of the baroreflex.

HRV biofeedback has already been shown to be a promising intervention for mental health disorders. For instance, researchers have found that HRV biofeedback can lead to significant reduction in depressive symptoms and reduce substance craving among individuals with chronic substance misuse (Zucker, Samuelson, Muench, Greenberg, & Gevirtz, 2009). Studies have also shown that HRV biofeedback treatment can provide significant relief for individuals with combat related PTSD (Tan, Dao, Farmer, Sutherland, & Gevirtz, 2011; Zucker et al., 2009). Individuals with PTSD are able to alleviate symptoms (e.g., hyperarousal) through HRV biofeedback training, by controlling and maintaining their respiration at resonant frequency. Consequentially, these individuals are able to produce large increases in both HRV and baroreflex gain, which increase parasympathetic activity and dampen the hyperactive autonomic system inherent in PTSD.

Relationship Between HRV and Factors of Psychological Resilience

There have been some supporting literature for the association between HRV and various psychological variables of resilience, including attachment theory correlates of attachment anxiety and avoidance, as well as personality profiles known to confer resilience.

HRV and attachment theory. Attachment theory has been found to play a role in stress

resilience (Barcons et al., 2014; Shibue & Kasai, 2014). Briefly, attachment theory proposes that an infant's desire for proximity to his or her attachment figure, or caregiver, is a biological drive, rather than a learned behavior (Bowlby, 1969). Given this basic need for proximity, attachment behavior can be understood as a set of learned, adaptive behaviors (e.g., crying, searching) to maintain optimal proximity with the attachment figure. Adult attachment theory further explains how this fundamental need becomes manifested in adulthood (Ainsworth, 1989). Expectations and responses to interpersonal situations that are learned in early life relationships, lead to the formation of enduring, internal working models (i.e., adult *attachment styles*) that guide patterns of intimate, interpersonal relationships. Although the infant attachment system modulates goals such as physical proximity and soothing in response to distress, the adult attachment system modulates cognitive and behavioral patterns related to proximity and intimacy within relationships (e.g., trust, dependency).

The adult attachment system has been conceptualized in terms of two primary dimensions of attachment style—attachment anxiety and attachment avoidance (Brennan, Clark, & Shaver, 1998). Attachment anxiety is characterized by intense feelings for and preoccupation about the partner, fear of loss and abandonment, and doubts about one's worth as a relationship partner. On the other hand, attachment avoidance is characterized by relative deactivation of attachment and distress signals, distrust of intimacy, reluctance to trust and rely on others, and preference for autonomy. High scores on either attachment dimension are described as indicating attachment insecurity. In addition, attachment anxiety and avoidance can be conceptualized in terms of self-concept (i.e., positive or negative thoughts about the self) and other-concept (i.e., positive or negative thoughts about others), respectfully (Bartholomew & Horowitz, 1991).

Attachment appears to be formative in the development of both intrapersonal and interpersonal resilience factors. Through the mechanism of self-concept, attachment appears to foster intrapersonal resilience factors. For instance, self-concept has been found to be closely related with resilient traits such as self-esteem and self-efficacy (Jacobs, Bleeker, & Constantino, 2003; Judge, Erez, Bono, & Thoresen, 2002; Troisi, Massaroni, & Cuzzolaro, 2005). Furthermore, via self-concept, attachment appears to foster other resilience factors, including: emotional and behavioral regulation (Leary, Schreindorfer, & Haupt, 1995); adjusting to major life transitions and relational conflicts (Lopez & Brennan, 2000; Lopez & Gormley, 2002). Also, through the mechanism of other-concept, attachment appears to develop interpersonal resilience factors. For instance, attachment appears to be closely associated with having healthy social support, a well-known resilience factor (Ozbay et al., 2007). Attachment appears to affect functional (i.e., quality) and structural (i.e., quantity) aspects of social support via the development of interpersonal skills and social competencies (Mallinckrodt & Wei, 2005). Individuals with positive attachment likely have strong support systems because they have social competencies; therefore, have higher levels of resilience (Karreman & Vingerhoets, 2012).

Each attachment dimension has been found to exhibit different stress responses. Previous studies have shown that attachment anxiety is often associated with self-reported distress related to medical (Ciechanowski, Walker, Katon, & Russo, 2002; Kotler, Buzwell, Romeo, & Bowland, 1994) and psychiatric symptoms (Maunder, Lancee, Nolan, Hunter, & Tannenbaum, 2006; Mikulincer, Florian, & Weller, 1993). On the contrary, attachment avoidance has been found to have less or no associations with self-reported distress (Ciechanowski et al., 2002; Mikulincer et al., 1993; Maunder et al., 2006), nor associations with other types of distress expressions (e.g., somatization or hostility; Mikulincer et al., 1993). These differential stress responses are

consistent with attachment theory suggesting that attachment anxiety develops as a result of reinforced perception of self being unworthy, fragile, and vulnerable, and reinforced interpersonal values of signaling distress in order to maximize proximity to attachment figures. Contrarily, attachment avoidance develops as a result of reinforced perception that others are not trustworthy or generally accessible, and reinforced interpersonal values of relative deactivation of distress signals. Moreover, attachment systems appear to develop individuals' capacities for stress and emotional regulation (Mikulincer, Shaver, & Pereg, 2003).

Although there has been very little study on HRV and attachment, studies examining the associations between attachment systems and stress reactivity in biological stress response systems (e.g., autonomic nervous system) suggest there is a relationship. Diamond & Hicks (2005) investigated whether perceptions of attachment security and current attachment styles were associated to vagal activity, which was assessed by resting levels of respiratory sinus arrhythmia. They found that vagal activity was positively associated with perceptions of attachment security, but negatively associated with overall attachment anxiety. In addition, they also found that men with higher perceptions of security in their current relationships more quickly returned to baseline levels after exposure to laboratory-based anger induction. In another study, Maunder et al. (2006) found that although attachment avoidance was not associated with self-reported distress, it was however inversely associated with HF HRV. This finding suggested that attachment avoidance was associated with attenuated vagal activity. Other studies have found similar patterns where attachment avoidance was associated with heightened ANS reactivity (i.e., elevated heart rate and blood pressure), but not with self-reported distress (Carpenter & Kilpatrick, 1996; Feeney & Kirkpatrick, 1996; Youngmee, 2006). The overall pattern of results across these studies support that attachment anxiety and avoidance are

associated with heightened autonomic reactivity. Such findings are consistent with the notion that attachment insecurity is associated with deficits in emotion regulation (Allen & Miga, 2010; Mikulincer et al., 2003). Furthermore, attachment systems appear to be implicated in individual differences of autonomic capacities for effective emotion regulation. It is perceivable then to hold HRV as one biological mechanism that governs attachment systems.

HRV and personality. Individual personality traits are potential factors that confer resilience. The prominent, widely accepted five factor model, purports that personality traits can be categorized in one of five dimensions—Conscientiousness, Agreeableness, Neuroticism (reversely known as Emotional Stability), Openness to experience, and Extraversion (Costa & McCrae, 1992). According to this model, Conscientiousness is the tendency to be organized, self-disciplined, dependable, and competent; Agreeableness reflects altruism, cooperation and sensitivity towards other, and the ability to be trusting and compassionate; Neuroticism is the tendency to experience negative emotions such as anxiety, depression, or anger, and represents overall vulnerability to stress and emotional instability; Openness to experience is characterized by having curiosity, general appreciation for art, intellectual curiosity, active imagination, varied opinions and beliefs, and openness to emotions; Extraversion represents gregariousness, having a breadth of activities, excitement seeking, and gaining energy from engaging with the external world. Previous studies have supported the link between these personality factors with stress resilience (Caska & Renshaw, 2013; Davey, Eaker and Walters, 2003; Riolli, Savicki and Cepani, 2002).

In addition, studies have shown that there is a strong link between personality and physical health, specifically cardiac health. Personality has been found to influence health either by influencing the probability of adopting healthier lifestyles (Raynor & Levine, 2009; van de

Bree, Przybeck, & Cloninger, 2006; Zohar & Cloninger, 2011) or moderating how one deals with stressful situations (Uliaszek et al., 2012; Zuidena et al., 2011). Furthermore, the five factor personality traits have also been associated with positive health outcomes. For instance, low Neuroticism and high Conscientiousness have been correlated with adaptive health behavior (Lodi-Smith et al., 2011), cardiac health (Chapman & Goldberg, 2011), and longevity (Chapman, Fiscella, Kawachi, & Duberstein, 2010).

Studies have also investigated the link between personality and autonomic functioning. Increased Neuroticism has been found to be linked to increased sympathetic activity (Drabant et al., 2011). Also, individual differences in the five factor personality traits were linked to individual differences in electrocardiogram (ECG) amplitude patterns, specifically high Neuroticism (Koelsch, Enge, & Jentschke, 2012). Although HRV studies related to personality have been very minimal, Zohar, Cloninger, & McCraty (2013) have investigated the correlates of personality traits on HRV. They found that Openness was negatively correlated with many of the HRV variables, including those indicative of lower sympathetic activity and those indicative of low parasympathetic activity. The low activity for both branches of the autonomic nervous system suggests that HRV is only weakly regulated in people high in Openness, but parasympathetic regulation is relatively greater than sympathetic influences (i.e., correlation with LF/HF was negative).They also found that Agreeableness was positively correlated with HRV HF, suggesting associated with parasympathetic activity. Furthermore, these studies appear to support that personality traits may confer resilience via the biological mechanism of HRV.

Purpose of the Current Study

Research has shown that HRV reflects the degree to which cardiac activity can be modulated in the face of changing situational demands. High HRV has been considered adaptive

given that much support has been found for its relationship with greater capacities to regulate stress, emotional arousal, resistance to stress, and positive emotions (Bornstein & Suess, 2000; Porges, Doussard-Roosevelt, Portales, & Greenspan, 1996). Low HRV, contrarily, has been considered maladaptive given consistent findings of its relationship with pathophysiology (Berntson et al., 1996; Dekker et al., 2000) and with several psychological disorders (Carney et al., 2000; Lakusic, 2007; McCraty, Atkinson, Tomasino, & Stuppy, 2001; Rechlin, Weis, Spitzer, & Kaschka, 1994). Given the use of HRV as an outcome measure of autonomic resiliency, the current study was undertaken in order to examine whether HRV can also predict psychological variables of resilience—general stress resiliency, attachment security, and resilient personality factors.

Hypotheses

Hypotheses for the current study are as follows:

1. The degree that individuals exhibit lower LF HRV, higher HF HRV, and lower LF/HF HRV would relate to a higher perception of overall stress resilience.
2. The degree that individuals exhibit lower LF HRV, higher HF HRV, and lower LF/HF HRV would relate to lower attachment anxiety and avoidance.
3. The degree that individuals exhibit lower LF HRV, higher HF HRV, and lower LF/HF HRV would relate to a stronger degree of extraversion, conscientiousness, agreeableness, emotional stability, and openness to experience.

Method

The current study was part of a larger, ongoing study by a large Southern California research university to investigate stress resilience among United States service members with the use of virtual environments (VE).

Participants

Twenty six male participants, recruited from the Army National Guard Special Forces, completed HRV measures. Study participants were recruited via email, and participated under informed consent in accordance with Fuller Graduate School of Psychology institutional review board requirements. After arriving back from a military deployment, 23 of 26 participants (88%) completed various psychological measures. Given that the study's purpose was to explore how HRV predicted post-deployment psychological variables, only participants who completed both HRV and psychological measures were observed. Participants ranged from ages 26-56 years old, with the mean age being 42.11 years old. Regarding race, 19 reported as being White or Caucasian (82.6%), one reported as being African American (4.3%), two reported as being Hispanic (8.7%), and one reported as being Asian (4.3%). As for education, five reported having "some college" (21.7%), two reported having 2-year college degree (8.7%), eight reported having a 4-year college degree (34.8%), six reported having a master's degree (26.1%), one reported having a doctoral degree (4.3%), and one reported having a professional degree (4.3%). Regarding gender, 22 participants self-identified as male (95.7%), and one participant self-identified as female (4.3%). Eighteen out of 23 participants reported they had prior deployment experience before participating in the study. Also, four participants were diagnosed with either PTSD, depression, or traumatic brain injury (17.4%), and one participant had a co-morbid diagnosis of PTSD and depression (4.3%).

Procedure

A computer-based questionnaire was administered to members of the Army National Guard Special Forces. Before taking part in the study, participants were informed that participation was completely voluntary and were asked to provide informed consent. Participants

were told that their responses would be confidential. They were informed that they would be rating a series of statements about certain behaviors and characteristics to see how well each one described their current state. They were also informed that they would be providing demographic and background information. Participants were cautioned that some of the prompts would include questions about traumatic events that they may have experienced. Participants were given contact information in order to ask questions or express concerns related to the study.

Next, participants were then fitted with the BIOPAC MP150, a chest-mounted device which measured electrocardiogram (ECG), respiration, and galvanic skin response (GSR). Following that, participants were then equipped with a variety of devices that served to enhance virtual reality experience. For example, participants were equipped with a Sony HMZ-T1H head-mounted display (HMD) fitted with an Intersense IntertiaCube^2 and a neoprene HMD "shield" to remove any peripheral visual stimuli not associated with the VE. During the first two combat scenarios, while participants used a Logitech Game Pad F510 to drive a Humvee, they were also placed on a tactile transducer floor to make the VE-related stimuli more life-like. During the final two scenarios, participants carried a mock M16A1 rifle to increase VE immersion. Scenarios were run on an Intel Core i7-2600 8-core CPU running at 3.4 GHz with 8GB of RAM using two nVidia GeForce GTX570 graphics cards in an SLI configuration.

After being fully equipped with psychophysiological and virtual reality equipment, participants engaged in a 2-minute physiological calibration period. During the first minute, participants were exposed to a black period which consisted of complete silence and darkness. The first minute was used to determine participants' resting heart rate, heart rate variability (HRV), respiration, and galvanic skin response (GSR). At approximately the 1-minute mark, participants were subjected to strong white noise to ascertain any changes in their physiological

responses from a startle stimulus. The final minute was like the first, but utilized as recovery time for participants to return to baseline physiological levels before engaging in the first VE scenario. After both groups of participants engaged the first two combat scenarios, the game pads were exchanged with mock rifles which then were used in the final two scenarios. Prior to the start of the third scenario, participants experienced another 2-minute calibration period identical to the first encounter.

Measures

Stress resilience. The Connor-Davidson Resilience scale (CD-RISC; Connor & Davidson, 2003) comprises of 25 items, each rated on a 5-point scale for how much a particular item was true for the participant, with anchors of 0 (*Not true at all*) to 4 (*True nearly all of the time*), giving a maximum possible score of 100. Higher scores reflect greater resilience.

Attachment styles (self-concept and other-concept). The Relationship Questionnaire (RQ; Bartholomew & Horowitz, 1991) consists of four short paragraphs, each describing a secure, fearful, preoccupied, or dismissive style of relating to others. Respondents indicate the degree to which they feel that each description matches their relationship style on seven-point Likert-type scales from 1 (*Disagree Strongly*) to 7 (*Agree Strongly*). The RQ has been used widely and scores have been found to be moderately stable (e.g., Scharfe & Bartholomew, 1998), although the transparent nature of the instrument is thought to bias responses in some cases (Yusof & Carpenter, 2012). In addition, the strength of attachment avoidance was measured by adding ratings for the fearful and dismissing attachment patterns and subtracting ratings for secure and preoccupied attachment patterns, based on a model presented by Scharfe and Cole (2006). Similarly, the strength of attachment anxiety was measured by adding ratings for the fearful and preoccupied attachment patterns and subtracting ratings for secure and dismissing

attachment patterns.

Personality. The Ten-Item Personality Inventory (TIPI; Gosling, Rentfrow, & Swann, 2003), a ten item scale, has participants rate how well pairs of personality traits represent them. Each item is rated as to how well it seems to characterize the participant, 1 (*Disagree Strongly*) to (*Agree Strongly*). Combined pairs of items provide subscales for Extraversion, Agreeableness, Conscientiousness, Emotional Stability (reverse score of combined item pair related to Neuroticism), and Openness to Experiences.

Psychophysiological data. Data for HRV was obtained according to the guidelines set forth by the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (1996) using frequency analyses, as this has been considered the best way to determine HRV from relatively short (approximately 5 minute) recordings of ECG data. HRV data was obtained using the BIOPAC MP 150 which had channels for GSR (GSR100C), ECG (ECG100C), and respiration (RSP100C).

All recording times, with the exception of the baseline and startle times were 2 minutes. Baseline and pure startle recording times were 1 minute each. Data were extracted using an in-house custom designed MATLAB program. HRV data was visually inspected for artifacts and ectopic beats using Acqknowledge software. All such artifacts were either deleted or replaced using cubic splicing, depending on suitability of surrounding data (Task Force, 1996). The recordings then underwent fast- Fourier transformation for power spectrum analysis. Transformed data was visually inspected for outliers. One outlier was identified and changed to the average of the other scores in that recording period. All power spectrum analyses were performed with data expressed in normalized units, per Task Force (1996) recommendations.

Virtual Environment modules. Participants were led through four virtual environments (i.e., combat scenarios) by a group of virtual guides. Combat sessions were variable in time length.

Combat Scenario 1. Participants were introduced to his or her role as the driver for the team and was instructed to drive a Humvee down a dirt road in Afghanistan with the rest of the team. To keep participants engaged in the virtual environment, participants had to maintain a specific distance between vehicles in the front and rear. The mission was to drive into a town to gather information. Prior to reaching that town, the convoy was halted after a male body was identified in the middle of the road. After realizing that the man was still alive, participants listened to a discussion between other team members about whether to help the man or not given the possibility of a booby trap. Upon the arrival of the Explosive Ordnance Division (EOD) to determine the presence of explosives, the man dies. The scenario continued with the EOD sending a robot to investigate the body. At this point, those in the mentor group were taken to a mentor session. However, participants in the non-mentor group continued with the scenario and learned that the body was indeed booby trapped. A large explosion soon followed which engulfed the robot. Participants then continued directly into Combat Scenario 2.

Combat Scenario 2. As the convoy continued into the town, the convoy commander discussed his concern about possible lookouts. As the participant was driving to exit town, a roadside explosion occurred which damaged the participant's Humvee. Subsequently, the gunner in the Humvee was rendered unconscious and the rest of the characters in the scenario reacted in various states of anxiety and panic. Those in the mentor group were led into Mentor Session 2. Those in the non-mentor group changed their equipment and began Combat Scenario 3.

Combat Scenario 3. After changing equipment and a 2-minute black period, participants were introduced to a new role as being part of a walking patrol in a city with the previous convoy team. To keep participants engaged in the virtual environment, participants were instructed to stay behind the team leader and visually inspect rooftops, windows, and doorways for possible threats. After walking a short distance, the team came across a woman being physically assaulted by several men. The team became angry and the team leader instructed their interpreter to stop the men. Though the team leader was displeased, he instructed the interpreter to tell the men that the beating had gone for too long and that they should let the woman go into exile if that was their custom. At this point, the non-mentor group continued to walking patrol in Combat Scenario 4. However, the mentor group began the next mentor session.

Combat Scenario 4. Participants entered into an Afghanistan marketplace with the same team in order to speak with a civilian elder. The elder became irate, telling the team they had to leave immediately. The surrounding crowd then began yelling at the team. As discussion with the elder continued, a civilian boy kicked his soccer ball into one of the participants' team members. The team member kicked the ball back, but accidentally into an alley. While the civilian boy chased after the ball, he tripped on a pressure-plated Improved Explosive Device (IED) that was meant to damage the team. Despite the best efforts of the participants' squad, the boy succumbed to his severe injuries. After participants witnessed the team leader trying to console and convince the team member who kicked the ball that it was not his fault, the boy's mother appeared crying over the boy's body. Other than participants in the mentor group, all others entered into a 2-minute black period.

Analytic Strategy

Data was analyzed using SPSS version 19. Hierarchical regressions were used to determine if HRV would be able to predict resilience outcomes. There were three independent variables—LF HRV, HF HRV, and LF/HF HRV ratio. There were eight dependent variables (i.e., resilience outcomes) of interest—one of which was overall stress resilience; five of which were personality traits of Extraversion, Agreeableness, Conscientiousness, Emotional Stability, and Openness to Experiences; two of which were attachment factors of self-concept and other-concept. For each resilience factor, the order of entry was as follows. At Step 1, the demographic characteristics of age, race, education, prior deployments, and psychiatric diagnoses were entered into the model. At Step 2, all resilience outcomes were entered.

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